INTRODUCTION

Nitrogen is an ecologically important nutrient in freshwater and marine systems, and in estuaries is commonly a limiting nutrient for phytoplankton production (Nixon, 1995; Pinckney et al., 2001; Ryther and Dunstan, 1971). Total nitrogen (TN) consists of many compounds, including organic nitrogen (ON) and inorganic nitrogen (IN). Concentrations of dissolved IN (DIN) have escalated due to increasing human populations (Middelburg and Nieuwenhuize, 2001), which in turn have increased development and intensified agricultural and livestock operations. Enhanced DIN loads may engender ecological impacts such as changes in biological communities and amplified primary production (Herbert, 1999; Middelburg and Nieuwenhuize, 2001). Dissolved inorganic nitrogen in estuaries may be dominated by nitrate (NO_3^-), or ammonium (NH_4^+) depending on the sources of nitrogen (N) to the system (Middelburg and Nieuwenhuize, 2001). Ammonium is of critical importance for understanding estuarine biogeochemistry because it is a reduced form of N preferred by phytoplankton (Gilbert et al., 1982; Paasche and Kristiansen, 1982; Pennock, 1987; Wheeler et al., 1982). In addition, NH_4^+ plays an important role in sustaining bacterial productivity by processes such as nitrification (Lipschultz et al., 1985; Pennock, 1987; Wofsy, 1983; Wofsy et al., 1981).

The total dissolved nitrogen (TDN) pool is thought to contain a large portion of dissolved ON (DON) (Burdige and Zheng, 1998), but little is known about its interactions and fluxes through estuaries because few studies have made direct measurements of DON (Bronk et al., 1994). This is partly because of analytical difficulties (Burdige and Zheng, 1998) and partly because only a fraction of DON

compounds has been identified (e.g. amino acids and urea; (Bronk et al., 1994)). As a result, the DON pool has usually been neglected in discussions about N cycling and primary production (Bronk et al., 1994; Burdige and Zheng, 1998). Several researchers have reported that the largest identified component of water column DON is dissolved amino acids (Cowan and Boynton, 1996; Keil and Kirchman, 1991; Yamashita and Tanoue, 2003) and that the same might be true in sediments (Cowan and Boynton, 1996).

Sediment-water exchange is an integral part of the nitrogen cycle and is greatly influenced by organic matter deposition and remineralization. Sedimentary processes such as ammonification, nitrification, and denitrification are driven by microbially-mediated processes and can result in complex interconversions between various nitrogen species. In addition, amino acids from organic matter are easily degraded by benthic bacteria resulting in NH_4^+ production (Herbert, 1999). Jorgensen et al. (1981) reported that deamination of amino acids in organic matter accounted for up to 25% of the NH_4^+ regenerated in sediments inhabited by certain organisms.

Relevance

A rapidly growing population in southeastern North Carolina (an estimated 18% growth over 13 years, (NCDWQ, 2000)) is putting increasing pressure on the Cape Fear River estuary (CFRE) from residential, agricultural and industrial development. North Carolina permits 280 wastewater discharges into the basin, with approximately 24% of the land used by intensive agriculture and livestock production, mostly swine and poultry (Mallin et al., 2003). As seen in several estuarine systems, one of the most alarming aspects of intensified development within the Cape Fear watershed is the escalating input

of nutrients in the estuary. In 2003, 20% of the estuary's monitored stations were considered to be negatively impacted by excessive nutrient loading (Mallin et al., 2003).

Nitrogen is especially of concern for the CFRE because TN loading to the river basin has changed considerably over the past 20 years. In the estuary in particular, average NH_4^+ concentrations have steadily increased over eight years (since the beginning of the monitoring record) (Figure 1), whereas NO_3^- (Figure 2a) and TN (Figure 2b) concentrations have not significantly changed.

Pathways

Delivery of nutrients, sediments and contaminants to estuaries is greatly influenced by watershed land use patterns (Dauer et al., 2000) and it is crucial for effective coastal-zone management to make accurate estimates of nutrient transport rates from land to sea (Uncles et al., 1998a; Uncles et al., 1998b). Nitrogen sources and input pathways to estuaries can be placed in two categories, point sources and nonpoint sources. Point sources consist of sewage outfalls, wastewater treatment plants, and storm water drains. Nonpoint sources include atmospheric deposition, runoff and riverine input, release from resuspended sediments, groundwater, and fluxes from bottom sediments.

Loading from nonpoint sources is dominant in the supply of new N in many watersheds, especially those with heavy agricultural activities, and as a result, nonpoint nutrient sources are a major concern for most estuaries (Pinckney et al., 2001). Accurate information about the net flux of nutrients through estuaries has been difficult to collect primarily because quantifying fluxes through the estuary depends not only on the nutrient

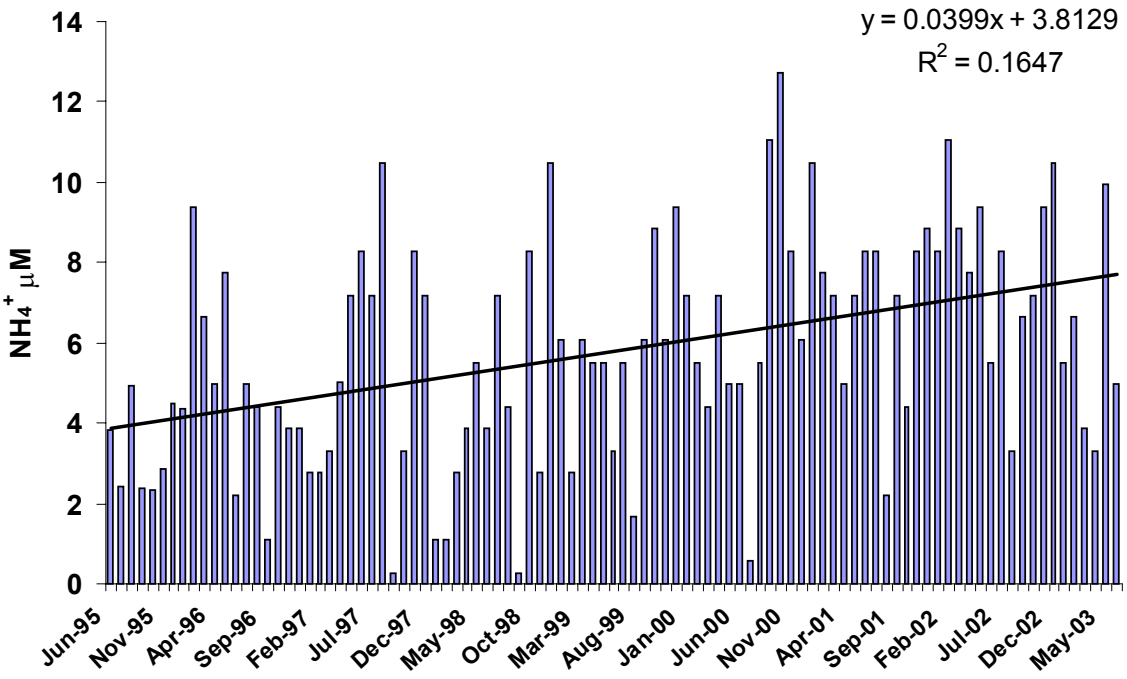


Figure 1. Concentrations of NH_4^+ from monthly data at M61 in the Cape Fear River Estuary from 1995 to 2003 showing a statistically significant increase (t-test, $p \leq 0.05$). Data from Mallin et al. (1996-2003).

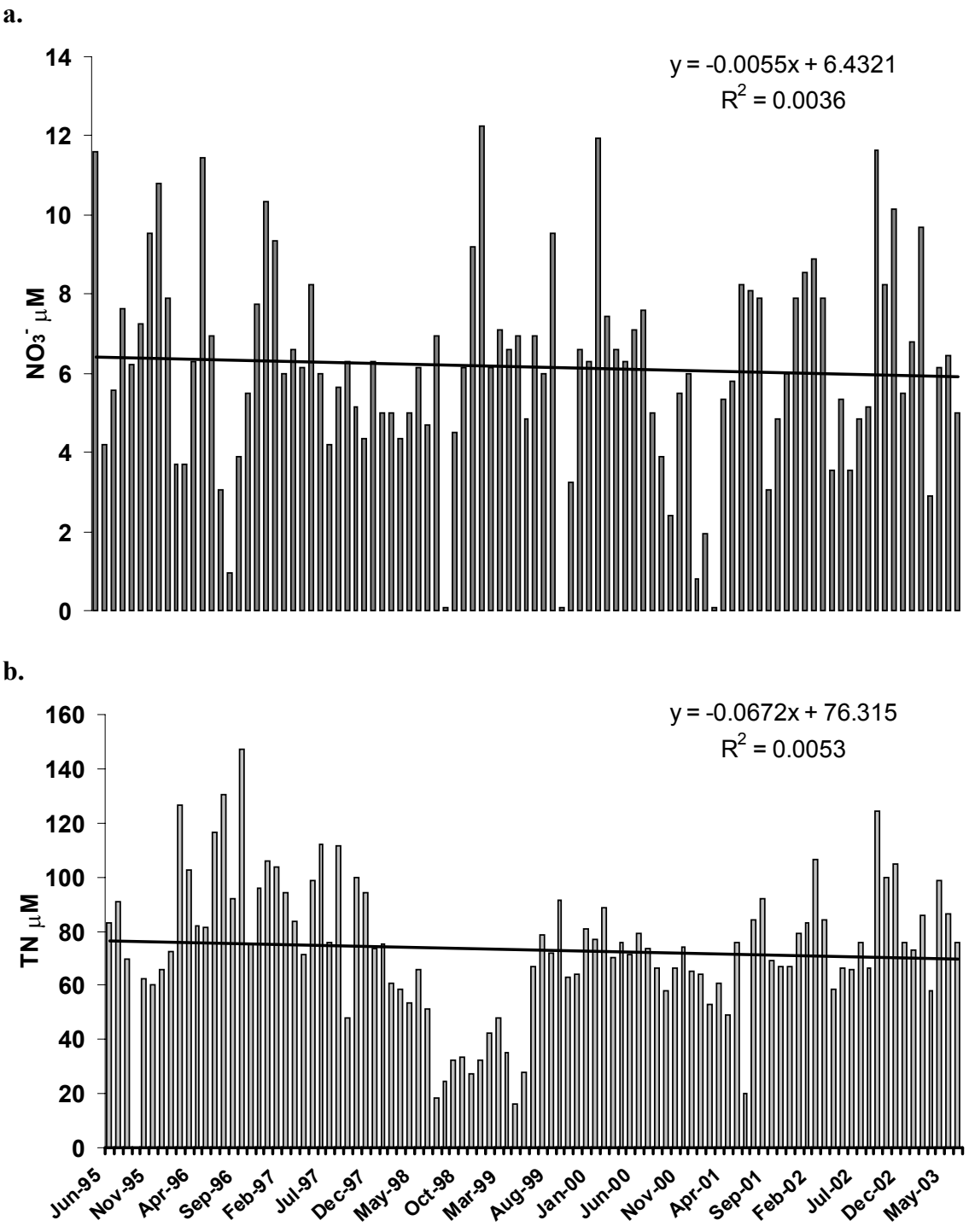


Figure 2. Concentrations of NO_3^- and TN from monthly data at M61 in the Cape Fear River Estuary from 1995 to 2003 showing no statistically significant change over the time period (t-test, $p \leq 0.05$). Data from Mallin et al. (1996-2003). **a.)** NO_3^- **b.)** TN

load, but also on the variety of complex and poorly understood transformation processes (Sanders et al., 1997).

Prior to this study, the magnitudes of individual pathways by which nitrogen is transported into the CFRE were not known. There are four major potential sources to consider for estuaries: riverine flow (mainly dependent upon rainfall and runoff), oceanic flux, atmospheric deposition, and sediments. Exchanges or fluxes can occur between any of these reservoirs. Other possible sources to consider are particle interactions, photochemical effects, sediment burial and biological processes (such as biological uptake, remineralization, nitrification, and denitrification) (Baird et al., 1995).

Each input must be quantified in order to understand its relative importance to the total loading and interconversions of nitrogen in the system. Transformations that occur between different forms of inorganic and organic nitrogen (NO_2^-, NO_3^-, NH_4^+, amino acids, DON) are also important to consider, and it is essential to quantify contributions of all nitrogen species in the CFRE to understand why NH_4^+ concentrations are increasing in the estuary.

Riverine Input

Increasing agricultural practices and wastewater flows have enhanced nutrient concentrations in riverine discharge to estuaries and coastal zones (Jordan et al., 1997). Nutrient concentrations in rivers and estuaries are directly related to nearby land uses: river catchments with intensive agriculture tend to have large NO_3^- concentrations in their estuaries, whereas rivers flowing from areas of small population and low agricultural intensity generally have small nutrient concentrations (Balls, 1994). On the eastern coast of the US for example, increasing anthropogenic inputs of N into the Chesapeake Bay

watershed have resulted in excessive phytoplankton production (Boynton et al., 1982; Correll, 1987; Fisher et al., 1992; Gallegos et al., 1992; Jordan et al., 1991; Malone et al., 1988; Malone et al., 1986) and increased episodes of hypoxic waters (Officer et al., 1984; Taft et al., 1980). River inputs are an important source of nutrients to estuarine and coastal areas (Uncles et al., 1998b), and the CFRE is a river-dominated estuary, so it is important to understand the magnitude of change and input of nutrients to these areas.

Atmospheric Deposition

Wet and dry atmospheric deposition is delivered to estuaries directly (landing on water surface) and indirectly (runoff from land) (Paerl, 1988; Whitall et al., 2003), and may be a significant contributor to total N loading and eutrophication in estuarine systems. The most common N species in atmospheric deposition are biologically active and include NH_4^+, NO_3^-, NO_2^- and DON, including amino acids and urea (Church, 1999; Duce, 1991; Mopper and Zika, 1987; Paerl, 1995; Peierls and Paerl, 1997; Russell et al., 1998; Timperley et al., 1985; Whitall et al., 2003). The most common form of DIN in wet deposition is NO_3^-, but NH_3 and NH_4^+ can be a significant portion of DIN (Buijsman et al., 1987; Whitall et al., 2003) when intensive livestock operations are nearby. Atmospheric deposition of NH_4^+ may be important for the CFRE in particular, because it is near intensive agricultural and livestock operations in the coastal plain region of southeastern North Carolina (Walker et al., 2000a; Walker et al., 2000b). A steadily increasing trend in atmospheric NH_4^+ deposition can be traced back to 1989 when a dramatic increase in swine and poultry production occurred in this region (Figure 3). While NH_4^+ deposition increased at Clinton, NC, the center of agricultural growth in the region, there was no significant change at Lewiston, NC (National Atmospheric

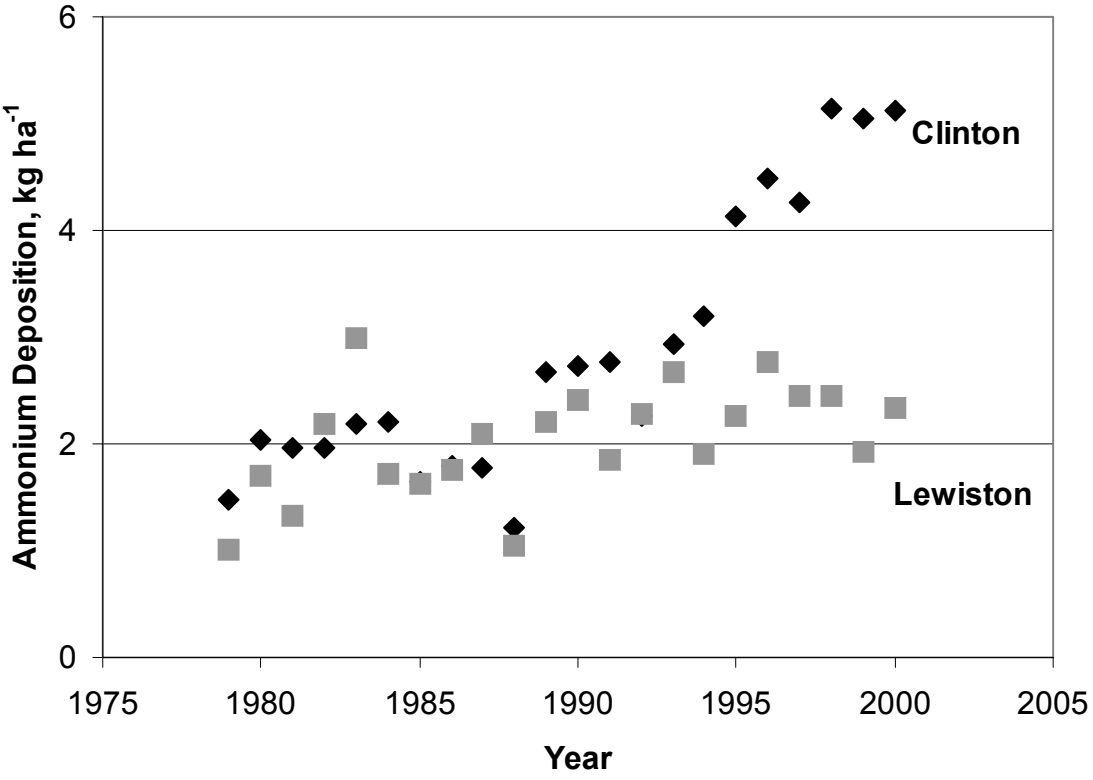


Figure 3. Atmospheric NH_4^+ deposition at Clinton and Lewiston, NC (NADP, 2004).

Deposition Program, 2004), located in a region of low agricultural activity. Atmospheric processes are considered a potentially significant source to the CFRE because the annual increase in water column NH_4^+ closely resembles the trend in atmospheric deposition of NH_4^+ at Clinton.

Benthic Fluxes

Shallow marine sediments (0.5 to 50 m water depth) encompass a significant reservoir of essential nutrients and play an important role in the estuarine nitrogen cycle (Blackburn and Sorensen, 1988; Carpenter and Capone, 1983; Herbert, 1999; Herbert and Nedwell, 1990; Sloth et al., 1995). Ammonium is often produced in sediments by the decomposition and deamination of organic matter by microbial organisms, and the concentration gradient that results typically leads to fluxes of NH_4^+ from sediments into overlying waters (Hammond et al., 1985; Kemp et al., 1990; Klump and Martens, 1987; Klump and Martens, 1989; Tzannis, 2000). Mallin et al. (1996, 2000) suggested that organic matter remineralization in Cape Fear estuarine sediments may be a potential cause of increased water column concentrations of nitrogen although no direct evidence for this has yet been presented.

Sediment Resuspension

Resuspended sediments have been implicated as a potential source of nutrients in estuaries. When sediments are suspended into the water column, nutrient-rich interstitial waters as well as sediment-associated nutrient species such as NH_4^+ become chemically and biologically available (Caetano et al., 1997; Uncles et al., 1998b). Morris et al., (1981, 1985) attributed a mid-estuarine maximum of NH_4^+ to its release from resuspended sediment within the high turbidity zone of the Tamar Estuary, England.

Peaks in water column NH_4^+ observed in the CFRE have also been tentatively attributed to sediment resuspension, particularly in the mid-estuary (S = 10-15) where there is a persistent turbidity maximum (Mallin et al., 1999b; Mallin et al., 1997-1998; Mallin et al., 1996). However, there has been no direct evidence to support this assertion.

Particles/Exchangeable NH_4^+

In addition to occurring in the dissolved form, a portion of the NH_4^+ produced in sediments can be adsorbed on particle surfaces, becoming unavailable for benthic fluxes (Van Raaphorst and Malschaert, 1995). Instead, rapid displacement can occur by physical and chemical processes such as resuspension, tidal flushing, ion exchange, and chemical diagenesis (Caetano et al., 1997; Mackin and Aller, 1984; Rosenfeld, 1979). Ammonium exchange is an important process to consider in estuarine systems because the process is both rapid and reversible, and readily occurs with the major cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) in seawater (Boatman and Murray, 1982); therefore particle-bound NH_4^+ may be a significant source to estuaries where freshwater first encounters saltwater. This may be especially important in the CFRE, where a persistent peak of NH_4^+ is present in the mid-estuary turbidity maximum (Mallin et al. 1996, 1998, 1999b). In this region NH_4^+ may be desorbed from resuspended sediments and particles as they encounter more saline seawaters.

Photochemical Production

Photochemical release of nitrogen from dissolved organic matter (DOM) may occur in organic-rich systems. The observed photoproduction of NH_3 and amino acids in natural waters (Bushaw et al., 1996; Li, 1996; Tarr et al., 2001; Wang et al., 2000) suggests that the incorporation of N into humics can have significant impacts on N

cycling in aquatic systems. When NH_3 is covalently bound to functional groups of humic acids, it is resistant to microbial attack or chemical degradation (Tarr et al., 2001). However, photochemical reactions may break these bonds, making NH_3 biologically available. Bushaw et al (1996) observed the production of NH_4^+ when humic-rich aquatic waters were exposed to sunlight, and research in the Cape Fear system has shown that photodegradation of humic substances can affect NH_4^+ production. There was a substantial increase in NH_4^+ concentrations when samples spiked with humic substances from the Cape Fear River were irradiated with sunlight (Li, 1996). It is therefore possible that, although the Cape Fear is light-limited, photochemical effects on nitrogen species in surface waters may be important.

Point Sources

Increases in direct discharge of wastewater treatment plants, and industrial and sewage outfalls may have a dramatic effect on the input of nutrients into aquatic systems. After evaluating N loadings to four East Coast U.S. estuaries, Whitall et al. (2004) determined that the TN load to two of the estuaries was dominated by human waste. The CFRE has many direct discharges into the system which could be implicated in associated increases in ammonium. Two important point sources in the CFRE near Wilmington include the Northside wastewater treatment plant which discharges treated water in the upper CFRE, and the Southside treatment plant which discharges into the middle estuary. There are several other discharges upstream of Wilmington, but they were not directly measured in this study.

Purpose

The goal of this study is to identify and quantify inputs of N species (TDN, NH_4^+, DON, amino acids and NO_3^-) into the Cape Fear River Estuary. Earlier N loading studies typically quantify any one or two of these pathways which yields an incomplete picture of N fluxes. This is a detailed estuarine study which takes a system-wide approach to determine N inputs to the system. An additional goal of the research presented here is to determine, based on the relative size of the fluxes, why NH_4^+ concentrations in the CFRE are increasing (Figure 1). Characteristics of the estuary and the ongoing development within the CFRE basin are similar to other worldwide estuarine systems. Therefore, we expect our observations and conclusions to be comparable to other estuaries and result in a better global understanding of N cycling in aquatic systems.

METHODS

Study Site

Cape Fear River Estuary (CFRE)

The Cape Fear River drains the largest river basin in North Carolina. Two coastal plain rivers, the Black and Northeast Cape Fear Rivers, contribute organic-rich freshwater to the estuarine portion of the mainstem river. The Atlantic Ocean as well as the Atlantic Intracoastal Waterway contribute seawater to the lower estuary. The estuary is generally well mixed vertically because of the relatively short residence time (typically 2-7 days) and rapid flow. It is comparatively shallow (1-2 m near the margins, and ~10-15 m in the main channel) and narrow. The CFRE is located between Brunswick and New Hanover counties in southeastern North Carolina and runs from north to south for

approximately 50 km from Wilmington, at the northernmost portion to Southport and Cape Fear, at the meeting point with the Atlantic Ocean.

Sampling stations from north to south in the estuary (Figure 4) were Navassa (located north of Wilmington), M61 (across from a state shipping port, just south of Wilmington), M54, M42, Station 1 (located between M42 and M35), M35, Station 2 (located near M23), M23, and M18 at the mouth of the estuary. Sediments were collected for core incubation experiments at M61, Station 1 and Station 2. M61 is located in the upper estuary and had an average salinity of 5 (range 0-10). The sediments at M61 were composed of soft, organic-rich mud with some whole plant material, and were characteristic of the upper estuary. Station 1 is located mid-estuary and had an average salinity of 12 (range 5-20). Sediments at Station 1 were mostly clay-rich mud with some sand. Station 2 is in the lower estuary and had an average salinity of 20 (range 15-27). Sediments were very sandy with only a small amount of mud, and with many shells.

Sample Collection and Storage

Water Column

Water column sampling was conducted on the R/V *Cape Fear*. Bottom and surface samples were typically collected using an air operated double-diaphragm Teflon® pump and Kynar tubing. Samples were also collected using a Seabird SBE 32 H20 Bottle Carousel and SBE 33 Real Time Deck Unit (Sea-Bird Electronics, Bellevue, WA, USA). Samples were filtered using trace metal clean 0.2 µm Meissner capsule filters and immediately analyzed for ammonium (NH_4^+) and free amino acids upon collection. Additional samples were collected in 125 mL high-density polyethylene (HDPE) bottles,

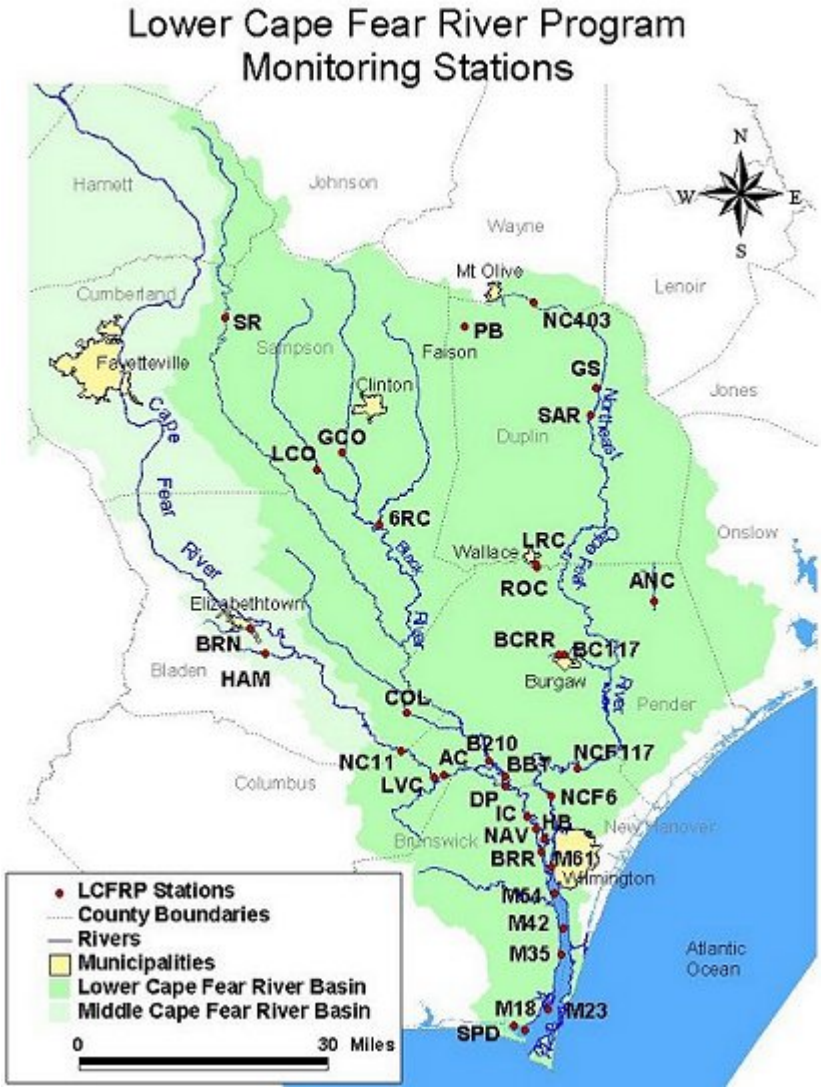


Figure 4. Cape Fear River Estuary sampling stations from Navassa to M18.

stored on ice until returned to the laboratory, then stored frozen (-20 ºC) until analysis for TDN, and $NO_2^- + NO_3^-$.

Bottom Sediments

Sediments were collected by lowering a box corer from the deck of the boat to the estuary bottom where it retrieved approximately 0.06-0.07 m^3 of relatively undisturbed sediment. Benthic flux samples were collected by subcoring a box core using four clear, acrylic core tubes at each site. The cores were 14 cm in diameter and 50-60 cm long. Plastic caps were placed on the top and bottom of the cores, and externally sealed with a manually tightened rubber gasket to prevent water leakage. The cores were transported back to the UNCW laboratory, where they were incubated at *in situ* temperature, and in the dark (to replicate ambient light and temperature levels). For exchangeable and photochemical resuspension experiments, three polypropylene cups were filled with the top 2-3 cm of bottom sediment at each site. The cups were sealed and placed in plastic bags on ice or refrigerated until used in the laboratory.

Suspended Particles

Suspended particles were collected once per season at all stations for exchangeable NH_4^+ experiments. Three liters (L) of unfiltered surface water was collected at each station. Samples of 200 mL were filtered through preweighed, precombusted Whatman GF/C glass filters (1.2 µm) and rinsed briefly with Milli-Q water to remove salts. At the minimum, triplicate filters were collected per station and individually placed in clean petri dishes, then stored on ice for use in the laboratory. A triplicate set of filtered particles was also collected to be dried and weighed in the laboratory for determination of suspended sediment concentrations.

Experimental Methods

Sediment Cores

Within 24 h of collection, three cores from each station were capped with a clear, acrylic cap fitted with tubing connected to a filtered air supply. They were also labeled with horizontal cm divisions along the side to keep track of water levels. Air was gently bubbled through to gently mix the water and to prevent overlying water from becoming stratified or anoxic. Cores were gently flushed with 2.5 L of unfiltered bottom water collected in 50 L carboys at each site. The water in these carboys was also used as the experimental control and for replacing water withdrawn from core incubations. Immediately after flushing the cores with carboy water, a sample was collected from all cores and both carboys to obtain an initial concentration (T0). During sampling, volumes of water removed from each core were immediately replaced with an equal volume of water from the appropriate carboy. The amount of water removed and replaced, and the time sampling took place were carefully recorded. The cores were sampled once a day for 4 to 5 days. All samples were filtered through trace metal clean 0.2 μm Meissner capsule filters into clean 125 mL HDPE bottles for immediate analyses of NH_4^+ and amino acids. The samples were then frozen for subsequent analyses of TDN, and $NO_3^- + NO_2^-$.

Exchangeable Experiments

Triplicate samples of the top 2-3 cm of sediment were collected at each station. Within 24 h of collection, sediments were homogenized individually and then homogenized into one sample for use in experiments. Sediments were separated and weighed into triplicate 50 mL polypropylene centrifuge tubes for porewater collection, and for modified KCl extractions as described by Rosenfeld (1979) and Mackin and Aller

(1984). For porewater collection, each tube was centrifuged at 3000 rpm for 10 minutes to separate pore water from sediments. The supernatant was removed and filtered through 0.45 μm Millipore filters. For KCl extractions, ~10 mL of 2 N KCl per g dry weight sediment were added to each tube. The dry weight of sediment samples was assumed to be 50% of the measured wet weight. To confirm this, a triplicate set of samples were weighed in aluminum pans, baked (at 60 ºC for 2-3 days) and weighed again to find % water of sediments from each station. Tubes of KCl extraction samples were vigorously shaken for one hour then centrifuged at 3000 rpm for 10 minutes and given the same treatment as the porewater set. Samples were immediately analyzed for NH_4^+ and dissolved free amino acids, and then frozen for further analyses if needed.

Exchangeable experiments were similar for filters containing suspended particles as for sediments. Each filter was placed in a 50 mL polypropylene centrifuge tube, 10 mL of 2 N KCl was added, the samples were then shaken, centrifuged, filtered and analyzed as described previously. A minimum (10 mL) of KCl solution was used with the filter extractions to avoid diluting exchangeable NH_4^+ with too much KCl. As a result, small samples were recovered and they were often not available for subsequent analyses.

Photochemical Production Experiments

Using bottom sediments and overlying water from each site, sediment suspensions were prepared at concentrations relevant to disturbed environments (1-2 g per L) (Schubel, 1975). Three polypropylene cups were filled with sediment at each site and homogenized into one sample to use for experiments. Controlled photolysis experiments were performed using procedures described in Kaczynski and Kieber (1993). Suspensions were divided into six 500 mL quartz flasks per treatment per station. A

sample was collected and set aside for immediate analysis of NH_4^+ and amino acids. Three quartz flasks were enclosed in black plastic bags to serve as dark controls. The three light flasks were placed in a constant temperature water bath (set at ambient estuarine temperature) and irradiated in simulated sunlight using a Spectral Energy™ solar simulator (1 kW Xe arc light source) with AM1 filter to remove wavelengths not found in the solar spectrum. The flasks were rotated every 3 h to minimize artifacts caused by different light exposures among the flasks. Dark and irradiated light flasks were filtered through 0.2 μm acid-washed Meissner capsule filters at the end of the experiment and analyzed for NH_4^+ and amino acids to determine photochemically-induced changes in concentrations.

Reagents and Standards

The working reagents (WR) used for NH_4^+ and free amino acid analyses were reagent grade materials obtained from Sigma Chemical Company (St. Louis, MO, USA) unless otherwise indicated. All standards were prepared from reagent grade materials. Deionized (DI) water was obtained from a Milli Q water system (18.2 MΩ resistance, Millipore, Bedford, MA, USA).

A 240 μM NH_4Cl standard was made by diluting a 0.5 M NH_4Cl solution in DI water. The NH_4^+ working reagent, as described by Holmes et al (1999) was a buffered solution of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) plus sodium sulfite ($NaSO_2$) and orthophthaldialdehyde (OPA, $C_8H_6O_2$) dissolved in 100 mL of HPLC grade acetonitrile obtained from Fisher Scientific (Fair Lawn, NJ). OPA is light sensitive and was prepared and stored in the dark. The complete reagent was stored in a large HDPE bottle covered

with aluminum foil to eliminate light degradation of the OPA. The final solution can be used after 24 h and is stable for up to 3 months when stored at room temperature (Holmes et al., 1999).

A 100 µM glycine standard was made by diluting a 10 mM glycine stock solution with DI water. The working reagent used in the amino acid (AA) analysis was also a buffered solution using sodium borate described by Parsons et al. (1984). The solution was adjusted to a pH of 9.5 ± 0.02 with NaOH, and mixed with OPA dissolved in HPLC grade acetonitrile and 2-mercaptoethanol (electrophoresis grade, Fisher Scientific). This solution was prepared and stored in the dark. The reagent can be used after 1 h and is good for 1 to 2 weeks when stored at 5°C in a dark HDPE bottle.

Analytical Methods

NH_4^+ and Dissolved Free Amino Acids

Ammonium in all samples was determined by using a modified version of a fluorometric method described by Holmes et al. (1999). The method is based on the reaction of OPA with NH_4^+, and in the presence of sulfite, blocks the fluorescent signal of free amino acids (Kerouel and Aminot, 1997). Free amino acids were measured by using a modified version of the fluorometric technique described by Parsons et al. (1984). OPA, in the presence of 2-mercaptoethanol, and without the required incubation time, reacts with free amino acids instead of NH_4^+. Free amino acids (AA) and NH_4^+ were determined using the standard additions method to eliminate analytical error due to matrix effects. Background fluorescence signal (BF) and working reagent blank (WRB) signals were subtracted from all fluorescence signals given by the instrument. For

example, when F_S = fluorescence signal of the sample, and $F_{S\text{-actual}}$ = the corrected fluorescence signal of the sample, then $F_{S\text{-actual}} = F_S\text{-BF-WRB}$. When F_{Std} = fluorescence signal of the standard addition, and $F_{Std\text{-actual}}$ = the corrected fluorescence signal of the standard addition, then $F_{Std\text{-actual}} = F_{Std} - \text{BF-WRB-}F_{S\text{-actual}}$. This corrects for fluorescence from the analytical reagent and colloids in the sample. When another medium was present in the samples, for example 2 M KCl from exchangeable experiments, a KCl blank was also measured and subtracted from the original sample signal along with the other blanks. From the above relation, the sample concentration can be determined by

$$C_{S\text{-actual}} = F_{S\text{-actual}} \times (C_{Std}/F_{Std\text{-actual}})$$

where C_{Std} is the concentration of the analytical standard.

Fluorescence for the NH_4^+ and amino acids was measured on a Turner Designs Model 450 Fluoremeter (Turner Designs, Sunnyvale CA, USA) with 360 nm excitation and 440 nm emission filters. For NH_4^+ analysis, triplicate samples and standard additions were prepared. Once the WR was added, the samples were allowed to incubate for 3 to 4 h in the dark before fluorescence was measured. Additional samples were pipetted and set aside for determination of the background fluorescence and working reagent blanks. They were analyzed at the same time as incubated samples, but the working reagent was not added until the blanks were ready for immediate fluorescence.

For amino acid analysis, triplicates were prepared of each sample and its standard addition. Three additional vials were filled with DI water to measure the working reagent blank (WRB). The fluorescence was recorded for the WRB vials and after a short incubation period for the sample and standard addition vials. Incubation time for amino

acids was the minimum time required for a sample to reach peak fluorescence. Incubation times varied between 2-4 min to reach peak fluorescence depending on sample type.

Total Nitrogen Determination

Total nitrogen was analyzed by the method of Alvarez-Salgado et al. (1998). High temperature catalytic oxidation (HTCO) measurements were performed using a commercial Shimadzu TOC-5050A coupled to an Antek 9000N nitrogen-specific chemiluminescence detector (Antek Instruments, TX, USA). Nitrate was used as the standard due to its good recovery (Merriam et al., 1996). A Hansel Laboratory Deep Seawater Reference (Lot # 06-00, Bermuda Biological Station for Research Inc.) was measured to confirm the accuracy of the analysis. Values obtained for the reference were 21.3 ± 0.24 μM N (n = 10) with accepted values ranging from 20.5 to 21.5 μM N (W.Chen, personal Communication).

Nitrate + Nitrite Determination

Nitrate + nitrite were analyzed on a Technicon Auto-Analyzer using an approved EPA method (Method 353.4). Duplicate samples are treated with ammonium chloride and passed through a copper treated cadmium column to convert the NO_3^- in each sample to NO_2^-. Nitrite reacts with a color reagent containing sulfanilimide and N-1 napthylethyleneidiamine dihydrochloride to form a pink colored dye. Color intensity is directly proportional to NO_x concentration in the sample. A colorimeter at wavelength of 550 nm is used to measure the dye intensity and displays the peaks on a chart recorder. Nitrate and NO_2^- standards were analyzed after every 10 samples for quality assurance. As part of the EPA protocol, reagent water blanks and random samples were spiked with a specific standard and analyzed for additional technique verification. Although both

NO_3^- + NO_2^- were measured, NO_2^- concentrations were insignificant, so the remainder of this thesis considers only NO_3^-.

Organic Nitrogen Determination

Organic nitrogen (ON) is determined by difference:

$$ON = TN - IN$$

(Cornell et al., 2003; Peierls and Paerl, 1997). Inorganic nitrogen (IN) is the sum NO_3^- and NH_x.

Detection Limits and Standard Error

Detection limits (DL) for NH_x, AA, and TN analysis were estimated from the standard deviation of replicates of a blank (Milli-Q water) taken after a complete incubation period. DL was determined from

$$DL = 3 \cdot S_o$$

where S_o is the standard deviation of the blank replicates. For NH_x, AA, and TN the DL was 0.17 μM (n = 10), 0.13 μM (n = 14), and 0.51 μM (n = 21), respectively (Long, 2003).

RESULTS

Benthic fluxes of nutrients were determined at three sites in the upper, middle and lower CFRE in November 2002; March, June, August, and November 2003; and February and April 2004. Seasons are divided up as winter (December, January, February), spring (March, April, May), summer (June, July, August) and fall (September, October, November). One experiment was conducted in winter, and two each in spring, summer and fall. Surface sediments were also collected at the same times for

exchangeable and photochemical experiments. Bottom and surface water samples were collected along the salinity gradient of the estuary ten times over nineteen months. Tables 1 and 2 show a summary of water column and sediment parameters measured at the time of sample collections.

Benthic Fluxes

Results from the core incubation experiments during each sampling period are presented in Table 3. There was significant seasonal and spatial variability in benthic fluxes of nitrogen species. Fluxes at M61, the upper estuary site with muddy, organic-rich sediment, always had larger fluxes of NH_4^+ compared to the other two stations. In addition, fluxes were always out of sediments at M61 (360 to 6100 $\mu mols \cdot m^{-2} \cdot d^{-1}$) whereas at Sta. 1 (-320 to 610 $\mu mols \cdot m^{-2} \cdot d^{-1}$) the flux was variable and at Sta. 2 (-100 to 20 $\mu mols \cdot m^{-2} \cdot d^{-1}$) the flux was usually into sediments. Average NH_4^+ fluxes from M61 and Sta. 1 were largest in winter (6100 to 610 $\mu mols \cdot m^{-2} \cdot d^{-1}$, respectively). Similarly to the NH_4^+ fluxes, NO_3^- fluxes were usually largest at M61 with the exception of fall, when the flux was larger at Sta. 1. However, NO_3^- fluxes were more variable (both in and out of sediments) at M61 (-600 to 1500 $\mu mols \cdot m^{-2} \cdot d^{-1}$) whereas at Sta. 1 they were always out of sediments (150 to 600 $\mu mols \cdot m^{-2} \cdot d^{-1}$). There were no significant measurable fluxes of NO_3^- at Sta. 2. Average annual estuarine NO_3^- fluxes were out of sediments (800 $\mu mols \cdot m^{-2} \cdot d^{-1}$).

Fluxes of DON were both in and out of sediments. Unlike NH_4^+ and NO_3^- there was no distinct difference between DON fluxes at M61 and the other two stations. DON fluxes were usually into sediments at M61 (-3100 to 500 $\mu mols \cdot m^{-2} \cdot d^{-1}$), of small

Table 1. Seasonal summary of water column parameters for Cape Fear estuarine transects. An X indicates no data available.

Date	Site	Temperature (ºC)		Salinity		DO (mg/L)	
		Surface	Bottom	Surface	Bottom	Surface	Bottom
Spring	Navassa	18.6	18.3	0.1	0.1	7.2	7.1
	M61	17.6	17.6	0.3	0.7	7.3	7.1
	M54	18.4	17.8	0.6	2.9	7.5	6.9
	M42	16.6	16.2	0.0	0.0	X	X
	St 1	15.0	15.2	1.1	4.6	7.8	7.9
	M35	18.6	18.4	2.5	13.4	7.7	7.3
	St 2	11.4	11.6	11.3	19.0	8.3	7.6
	M18	19.6	18.0	20.6	20.6	X	X
Summer	Navassa	25.5	26.0	0.0	0.0	3.4	3.2
	M61	26.4	26.5	0.0	0.2	3.4	3.2
	M54	27.1	27.0	0.0	0.0	3.6	3.3
	St 1	26.1	26.3	0.5	5.5	3.9	3.5
	M35	27.2	26.9	1.7	14.6	4.3	4.1
	Station 2	27.5	26.7	17.8	26.4	X	X
	M18	27.3	26.9	9.6	28.1	5.1	4.5
Fall	Navassa	X	X	0.0	0.0	X	X
	M61	17.0	X	6.5	X	6.5	X
	M54	17.4	X	8.3	X	6.5	X
	Station 1	16.6	17.2	5.7	14.0	7.0	6.7
	Station 2	17.4	17.5	14.5	14.7	7.4	7.4
	M23	18.5	X	24.9	X	7.4	X
Winter	Navassa	7.4	X	0.0	0.0	11.9	X
	M61	7.8	X	2.6	5.0	11.1	X
	M54	8.0	X	6.0	11.0	11.6	X
	Station 1	8.1	X	5.0	14.0	11.6	X
	M35	8.4	X	10.0	16.0	11.3	X

Table 2. Seasonal summary of sediment parameters for Cape Fear estuarine transects. An asterisk indicates cores collected for incubation experiments, and X indicates no data available.

Date	Site	Sediments		
		Description	% Water	% Organic Carbon
Spring	Navassa	Muddy, sulfidic, some plant material	66.9	8.3
	M61*	Soft, wet, fine mud	73.2	8.1
	M54	Soft, wet, fine, mud	24.4	2.5
	M42	Muddy sand	49.2	2.8
	Station 1*	Muddy sand, shells	50.1	5.3
	M35	Coarse, sandy mud	27.1	2.4
	Station 2*	Sand	29.1	4.5
	M18	Sand and shells	X	1.3
Summer	Navassa	Soft mud	64.6	6.0
	M61*	Soft, wet mud, sulfidic with some plant material	64.6	7.0
	M54	Sandy, sulfidic mud	25.8	0.7
	Station 1*	Fine muddy clay	41.5	6.1
	M35	Fine muddy sand and shells	55.9	1.1
	Station 2*	Sand and shells	X	X
Fall	Navassa	Soft wet mud	62.3	4.5
	M61*	Soft, wet, sulfidic mud	67.0	6.5
	M54	Coarse, sandy, sulfidic mud	27.7	1.3
	Station 1*	Sulfidic clay and mud	67.4	7.1
	Station 2*	Sand	31.8	X
	M23	Coarse sand, little mud	21.0	0.7
Winter	Navassa	Soft, wet, sulfidic mud	60.0	5.3
	M61*	Soft, wet, very fine mud	81.0	8.6
	M54	Sandy mud	70.5	6.1
	Station 1*	Sandy clay and mud	54.5	4.4
	M35	Muddy sand	50.3	2.2

Table 3. Seasonal benthic flux measurements for nitrogen species. Fluxes are averages in $\mu mols \cdot m^{-2} \cdot d^{-1}$. Non-statistically significant fluxes are represented as '0' net flux. A negative flux (-) represents an inward flux to sediments from the overlying water. An asterisk indicates the average is of seasons from different years. An X in the table represents no data available. Means and standard deviations (or range) for each constituent are indicated for each season. See Appendix A for a detailed summary of individual cores and standard deviations.

Season	Site	NH_4^+	NO_3^-	DON	Amino Acids	TDN
Spring	M61	360	1500	-680	5	1200
	Station 1*	41	450	190	5	680
	Station 2	-17	0	200	-5	180
	Mean	**130 ± 200**	**670 ± 790**	**-95 ± 500**	**2 ± 6**	**700 ± 520**
Summer	M61	3300	-380	-180	450	2700
	Station 1*	-57	150	-48	-18	47
	Station 2	-100	0	100	-29	0
	Mean	**1000 ± 2000**	**-75 ± 270**	**-40 ± 140**	**130 ± 270**	**920 ± 1600**
Fall	M61	400	470	500	46	1400
	Station 1*	-320	600	-96	0	180
	Station 2	20	X	X	X	X
	Mean	**33 ± 360**	**540 ± -70**	**200 ± 300**	**23 ± 23**	**780 ± 590**
Winter	M61	6100	-600	-3100	310	2400
	Station 1	610	160	10	34	780
	Station 2	X	X	X	X	X
	Mean	**3400 ± 2800**	**-220 ± -380**	**-1500 ± -1600**	**170 ± 140**	**1600 ± 810**
Annual	M61	10000	1000	-3500	810	7700
	Station 1	280	1400	57	21	1700
	Station 2	-100	0	300	-34	180
	Mean	**3400 ± 5800**	**800 ± -710**	**-1000 ± -2100**	**270 ± 470**	**3200 ± 4000**

magnitude and variable direction at Sta. 1 (-96 to 190 μmols·m^{-2}·d^{-1}); and of small magnitude and out of sediments at Sta. 2 (100 to 200 μmols·m^{-2}·d^{-1}). The average estuarine flux of DON was largest and directed into sediments in winter and was relatively small during other seasons.

Amino acid fluxes were similar to NH_4^+ but of smaller magnitude. Fluxes of amino acids at M61 were usually larger than at the other stations and were always out of the sediments (5 to 450 μmols·m^{-2}·d^{-1}). Station 1 fluxes were usually out of sediments (-18 to 34 μmols·m^{-2}·d^{-1}) whereas at Sta. 2 they were into the sediments (-29 to -5 μmols·m^{-2}·d^{-1}). Like NH_4^+, average estuarine fluxes of amino acids were largest in winter.

In this section, TDN measured by the HTCO method consists of DIN and DON. The species contributing to TDN fluxes were seasonally and spatially variable, however fluxes of TDN were out of sediments (47 to 2700 μmols·m^{-2}·d^{-1}) at all stations during all seasons. TDN fluxes at M61 were mainly due to NH_4^+ in summer and winter, NO_3^- in spring, and DON in fall. At Sta. 1 the major contributor was NO_3^- except in winter when NH_4^+ dominated fluxes. The only significant and measurable flux at Sta. 2 was in spring when DON was the majority of TDN. The largest average estuarine TDN flux occurred in winter (1600 μmols·m^{-2}·d^{-1}) when NH_4^+ was the dominant species fluxing out of M61 sediments and the smallest was in spring (700 μmols·m^{-2}·d^{-1}) when NO_3^- made up the largest portion of TDN.

Exchangeable Experiments

Surface sediments and suspended particles collected along the salinity gradient in the estuary were resuspended both with 2 M KCl to determine exchangeable NH_4^+ and

freshwater (FW) collected from Navassa to determine NH_4^+ and amino acid released by resuspension alone. Ammonium results from these experiments are shown in Table 4.

Upper estuary sediments typically released higher concentrations of NH_4^+ compared to middle and lower estuary sediments. Specifically, NH_4^+ released from M61 sediments (2 to 7 μmols $NH_4^+ \cdot g^{-1}$ dry sediment), located near the limit of seawater intrusion, was usually an order of magnitude larger than at other stations (0 to 5 μmols $NH_4^+ \cdot g^{-1}$ dry sediment). At most stations, KCl-treated sediments released at least 50% more NH_4^+ than those resuspended with FW (0 to 2 μmols NH_4^+ released$\cdot g^{-1}$ dry sediment), except in the lower estuary, where the difference between KCl and FW treatments became less obvious. This suggests that exchangeable NH_4^+ had already been released from sediments in the lower estuary. Ammonium released from suspended particles (14 to 300 μmols NH_4^+ released$\cdot g^{-1}$ particle) was always greater than that released from bottom sediments and did not vary with salinity.

Table 5 shows results of amino acids released during exchange experiments. Amino acids released from bottom and suspended sediments showed a similar pattern as seen with NH_4^+. Typically, as with NH_4^+, sediments from the upper estuary (0.1 to 0.7 μmols Gly released$\cdot g^{-1}$ dry sediment) released more amino acids than lower estuary sediments (0 to 0.1 μmols Gly released$\cdot g^{-1}$ dry sediment). KCl treatments released at least twice the concentration of amino acids than did FW, and suspended particles (1.7 to 23 μmols Gly released$\cdot g^{-1}$ particle) always released considerably more amino acids than bottom sediments. At least 15% more amino acids were released from susupended particles treated with KCl than with FW (10 to 19 μmols Gly released$\cdot g^{-1}$ particle).

Table 4. Average seasonal exchangeable NH_4^+ from bottom sediments and suspended sediments in surface water. An X indicates no data. Freshwater collected at the same time was used to resuspend sediments to see how much NH_4^+ is released from resuspension alone. Refer to Table 1 for salinity and temperature at the time of collection and Appendix B for a detailed summary of individual samples and uncertainties.

Date	Site	Sediments		Particles	
		µmols NH_4^+ released g^{-1} dry sediment		µmols NH_4^+ released g^{-1} particle	
		2 M KCl	FW	2 M KCl	FW
Fall	Navassa	3.0	1.0	14	X
	M61	2.0	0.4	85	X
	M54	0.3	0.1	63	X
	Station 1	1.0	0.2	42	X
	Station 2	0.0	X	X	X
	M23	0.0	0.0	20	X
Winter	Navassa	1.0	0.9	74	78
	M61	2.0	0.8	66	22
	M54	1.0	0.2	66	X
	Station 1	1.0	0.2	73	X
	M35	0.3	0.0	81	X
Spring	Navassa	0.1	0.0	18	X
	M61	7.0	2.0	22	X
	M54	0.5	0.3	38	X
	Station 1	0.6	0.0	32	X
	M35	0.0	0.0	32	X
	Station 2	0.1	X	X	X
Summer	Navassa	5.0	1.9	70	X
	M61	4.0	1.6	260	X
	M54	0.1	0.1	190	X
	Station 1	0.9	0.2	300	X
	M35	0.2	0.3	300	X
	Station 2	X	X	X	X
	M18	X	X	X	X

Table 5. Average seasonal exchangeable amino acids from bottom sediments and suspended sediments in surface water. An X indicates no data. One standard deviation is represented by ± for n = 3. Freshwater collected at the same time was used to resuspend sediments to see how much amino acids are released from resuspension alone. Amino acids were analyzed using a glycine standard, 'Gly' in the table represents glycine-equivalent units. Refer to Table 1 for salinity and temperature at the time of collection and Appendix C for a detailed summary of individual samples and uncertainties.

Date	Site	Sediments		Particles	
		µmols Gly released g^{-1} dry sediment		µmols Gly released g^{-1} particle	
		2 M KCl	FW	2 M KCl	FW
Fall	Navassa	0.2	0.1	3.8	X
	M61	0.1	0.1	19	X
	M54	0.0	0.0	3.4	X
	Station 1	0.1	0.1	3.5	X
	Station 2	X	X	X	X
	M23	X	X	X	X
Winter	Navassa	0.2	0.1	23	19
	M61	0.2	0.0	15	10
	M54	0.2	0.1	12	X
	Station 1	0.1	0.0	10	X
	M35	0.1	0.0	14	X
Spring	Navassa	0.1	0.0	3.1	X
	M61	0.7	0.2	1.7	X
	M54	0.1	0.1	8.3	X
	Station 1	0.0	0.0	5.2	X
	M35	0.1	0.0	4.2	X
	Station 2	X	X	X	X
Summer	Navassa	0.4	0.1	X	X
	M61	0.2	0.2	X	X
	M54	0.0	0.0	X	X
	Station 1	0.1	0.0	X	X
	M35	0.0	0.1	X	X
	Station 2	0.1	X	X	X
	M18	X	X	X	X

Photochemistry

Three treatments were used for the photochemical irradiation experiments: unfiltered water without sediment, filtered water without sediment and filtered water with sediment. Irradiation of both unfiltered estuarine waters and sediment suspensions did not generate concentrations of either NH_4^+ or amino acids that were distinguishable from the dark controls. Tables 6 and 7 show summaries of NH_4^+ and amino acid data from photochemistry experiments.

Atmospheric Fluxes

Atmospheric fluxes of nitrogen species to the Cape Fear River basin were calculated using data collected by Long (2003) (Table 8). Fluxes of NH_4^+ were smallest in winter and largest in spring (9.2 to 51 $\mu mols \cdot m^{-2} \cdot d^{-1}$). Nitrate fluxes (18 to 58 $\mu mols \cdot m^{-2} \cdot d^{-1}$) were largest in summer, and although the spring flux of NO_3^- was smaller than NH_4^+, in other seasons NO_3^- fluxes were usually considerably larger than NH_4^+.

Atmospheric fluxes of DON (0 to 18 $\mu mols \cdot m^{-2} \cdot d^{-1}$) were smaller than DIN fluxes, and unlike DIN, were largest in fall. Amino acids (1 to 4 $\mu mols \cdot m^{-2} \cdot d^{-1}$) were not as important as NO_3^- and NH_4^+, but similarly, were largest in spring. TDN fluxes were largest in summer (30 to 100 $\mu mols \cdot m^{-2} \cdot d^{-1}$) when NO_3^- was the major contributor to TDN.

Riverine Fluxes

The following equation was used to calculate riverine fluxes in moles per season of the various N species into the CFRE:

Table 6. Seasonal photochemical production of NH_4^+. Concentrations in µM. See Appendix D for a detailed summary of individual experiments and standard deviations. **a.)** Filtered with sediment **b.)** Filtered without sediment **c.)** Unfiltered without sediment

a.

Season	Site	Filtered with sediment			
		T0	Dark	Light	**Δ**
Spring	M61	1.6	5.6	6.2	**0.6**
	M54	1.6	1.5	1.9	**0.4**
	M42	0.9	0.8	0.6	**-0.1**
	M35	0.8	2.4	2.7	**0.3**
	M23	1.5	1.4	1.4	**0.1**
Summer	M61	3.1	5.6	5.2	**-0.4**
	Sta 1	3.1	3.8	5.5	**1.8**
	Sta 2	3.6	4.6	3.8	**-0.8**
Fall	M61	8.0	13	13	**0.3**
	Sta 1	5.2	7.3	7.7	**0.4**

b.

Season	Site	Filtered without sediment			
		T0	Dark	Light	**Δ**
Summer	M61	3.1	3.2	7.6	**4.4**
	Sta 1	3.3	3.1	3.7	**0.7**
	Sta 2	0.9	2.2	3.7	**1.5**
Fall	M61	8.0	6.9	7.9	**1.1**
	Sta 1	5.2	5.5	5.2	**-0.3**

c.

Season	Site	Unfiltered without sediment			
		T0	Dark	Light	**Δ**
Summer	M61	3.1	3.3	3.4	**0.1**
	Sta 1	3.2	2.9	3.7	**0.8**
	Sta 2	3.0	3.2	3.0	**-0.2**
Fall	M61	8.0	7.4	8.3	**0.9**
	Sta 1	5.2	4.1	6.0	**1.9**

Table 7. Seasonal photochemical production of amino acids. Concentrations in µM. See Appendix E for a detailed summary of individual experiments and standard deviations. **a.)** Filtered with sediment **b.)** Filtered without sediment **c.)** Unfiltered without sediment

a.

Season	Site	Filtered with sediment			
		T0	Dark	Light	**Δ**
Spring	M61	1.1	0.9	1.0	**0.2**
	M54	1.5	1.0	1.1	**0.1**
	M42	0.8	0.9	0.7	**-0.2**
	M35	0.9	1.0	0.9	**-0.2**
	M23	1.0	0.9	0.8	**-0.1**
Summer	Sta 1	1.8	1.5	1.4	**-0.1**
	Sta 2	1.7	11	5.3	**-5.7**
Fall	M61	2.9	3.6	3.2	**-0.4**
	Sta 1	1.7	1.8	2.1	**0.3**

b.

Season	Site	Filtered without sediment			
		T0	Dark	Light	**Δ**
Summer	Sta 1	1.6	1.9	1.3	**-0.6**
	Sta 2	0.9	1.0	0.8	**-0.2**
Fall	M61	2.9	3.5	3.1	**-0.4**
	Sta 1	1.7	1.6	1.2	**-0.4**

c.

Season	Site	Unfiltered without sediment			
		T0	Dark	Light	**Δ**
Summer	Sta 1	1.4	1.6	1.5	**-0.1**
	Sta 2	1.7	1.7	1.4	**-0.3**
Fall	M61	2.8	2.5	2.4	**-0.1**
	Sta 1	1.7	1.4	0.9	**-0.5**

Table 8. Seasonal summary of nitrogen species measured in atmospheric wet deposition to the Cape Fear River Estuary from 2001 to 2003 (Long, 2003). **a.)** Concentrations are volume weighted averages (VWA) in µM. Volume of precipitation collected is in mm. **b.)** Atmospheric fluxes in µmols·m^{-2}·d^{-1}.

a.

Season	Volume	NH_4^+	NO_3^-	DON	Amino Acids	TDN
Spring	385	12	8	0	1	17
Summer	675	5	8	1	1	13
Fall	405	4	5	4	1	13
Winter	107	8	15	2	1	25
Annual	**1572**	**8**	**9**	**2**	**1**	**18**

b.

Season	NH_4^+	NO_3^-	DON	Amino Acids	TDN
Spring	51	35	0.0	3	74
Summer	37	58	5	4	100
Fall	17	23	18	2	57
Winter	9	18	2	1	30
Annual	**32**	**38**	**7**	**3**	**77**

$$Mols \cdot season^{-1} = C\ (mols \cdot L^{-1}) \times Streamflow\ (m^3 \cdot sec^{-1}) \times 1000\ L \cdot m^{-3} \times 60\ sec \cdot min^{-1} \times 60\ min \cdot hr^{-1} \times 90\ (d \cdot season^{-1}) \times 24\ h \cdot d^{-1},$$

where C = average seasonal N concentration measured in the three tributary rivers by the Lower Cape Fear River Program (LCFRP; Mallin et al 2001, 2002, 2003) and *streamflow* = seasonal average of monthly river flow available from the United States Geological Survey – water resources (http://waterdata.usgs.gov/nc/nwis/sw). River discharge varied from 160 to 370 $m^3 \cdot sec^{-1}$ (Table 9).

River fluxes of nitrogen species were important to the estuary although the relative contributions from the three rivers varied. Fluxes of NH_4^+ were considerable in all seasons, and there was little variation (7 x 10^6 to 1 x 10^7 $mols \cdot season^{-1}$). Similarly, there was no seasonal variation in total riverine contribution of NO_3^- (1 x 10^7 to 2 x 10^7 $mols \cdot season^{-1}$); however there was a distinct difference between each river's contributions. The Cape Fear River (1 x 10^7 to 2 x 10^7 $mols \cdot season^{-1}$) had at least an order of magnitude larger NO_3^- flux than the other two rivers (7 x 10^4 to 1 x 10^6 $mols \cdot season^{-1}$). The largest difference was in fall, when the NO_3^- contribution was smallest from the Northeast Cape Fear and Black rivers. Fluxes of organic nitrogen (ON) were much larger than NH_4^+ and NO_3^- with the largest flux occurring in spring (1 x 10^8 to 2 x 10^8 $mols \cdot season^{-1}$). There was always a difference between the three rivers, but not as distinct as the NO_3^- fluxes. Total nitrogen (TN) data from the Lower Cape Fear River Program are from unfiltered samples, and is different from TDN mentioned in above sections. Total riverine fluxes of TN varied with season with largest fluxes occurring in spring and winter (1 x 10^8 to 2 x 10^8 $mols \cdot season^{-1}$). In all seasons ON was the major component of TN.

Table 9. Seasonal river contributions using data from Mallin et al. (2001, 2002, 2003) and USGS (2001, 2002, 2003). ON = organic nitrogen, TN = total nitrogen for unfiltered samples **a.)** Concentrations in µM **b.)** Fluxes in mols season^{-1}

a.

Season	River	NH_4^+	NO_3^-	ON	TN
Spring	Cape Fear	5	9	65	79
	NE Cape Fear	4	4	68	76
	Black	3	2	50	55
	Mean	**4**	**5**	**61**	**70**
Summer	Cape Fear	5	11	82	98
	NE Cape Fear	6	3	62	71
	Black	5	1	47	53
	Mean	**5**	**5**	**64**	**74**
Fall	Cape Fear	6	16	107	129
	NE Cape Fear	3	1	67	71
	Black	4	1	61	66
	Mean	**4**	**6**	**79**	**89**
Winter	Cape Fear	4	13	79	96
	NE Cape Fear	4	5	63	72
	Black	3	4	46	53
	Mean	**4**	**7**	**63**	**73**

b.

Season	River	Streamflow ($m^3 \cdot sec^{-1}$)	NH_4^+	NO_3^-	ON	TN
Spring	Cape Fear	309	1×10^7	2×10^7	2×10^8	2×10^8
	NE Cape Fear	31	1×10^6	1×10^6	2×10^7	2×10^7
	Black	33	7×10^5	6×10^5	1×10^7	1×10^7
	Total	**373**	$\mathbf{1 \times 10^7}$	$\mathbf{2 \times 10^7}$	$\mathbf{2 \times 10^8}$	$\mathbf{2 \times 10^8}$
Summer	Cape Fear	169	7×10^6	1×10^7	1×10^8	1×10^8
	NE Cape Fear	31	1×10^6	7×10^5	2×10^7	2×10^7
	Black	38	2×10^6	4×10^5	1×10^7	2×10^7
	Total	**238**	$\mathbf{1 \times 10^7}$	$\mathbf{1 \times 10^7}$	$\mathbf{1 \times 10^8}$	$\mathbf{1 \times 10^8}$
Fall	Cape Fear	131	6×10^6	2×10^7	1×10^8	1×10^8
	NE Cape Fear	13	3×10^5	7×10^4	7×10^6	7×10^6
	Black	12	4×10^5	8×10^4	6×10^6	6×10^6
	Total	**156**	$\mathbf{7 \times 10^6}$	$\mathbf{2 \times 10^7}$	$\mathbf{1 \times 10^8}$	$\mathbf{1 \times 10^8}$
Winter	Cape Fear	194	6×10^6	2×10^7	1×10^8	2×10^8
	NE Cape Fear	23	8×10^5	1×10^6	1×10^7	1×10^7
	Black	23	5×10^5	6×10^5	8×10^6	9×10^6
	Total	**240**	$\mathbf{7 \times 10^6}$	$\mathbf{2 \times 10^7}$	$\mathbf{1 \times 10^8}$	$\mathbf{2 \times 10^8}$

Wastewater Treatment

Wastewater discharge fluxes in moles of N species per season were calculated using data from the City of Wilmington Northside and Southside Wastewater treatment facilities and the following equation:

$$Mols \cdot season^{-1} = C\ (mols \cdot L^{-1}) \times Discharge\ (L \cdot d^{-1}) \times 90\ d \cdot season^{-1},$$

where *C* = seasonal average of monthly concentrations measured in discharge by the Northside and Southside treatment facilities and *discharge* = seasonal volume of average monthly discharge from the facilities. The only nitrogen species measured in wastewater discharge were NH_4^+ and total Kjeldahl nitrogen (TKN, which is NH_4^+ and ON); these data are summarized in Table 10. Fluxes of both NH_4^+ (6×10^6 to 7×10^6 mols·season^{-1}) and TKN (7×10^6 to 1×10^7 mols·season^{-1}) were largest in spring and summer. Organic nitrogen in wastewater discharge made up a small portion of TKN and was also largest in spring (2.7×10^6 mols·season^{-1}). The majority of TKN appears to be NH_4^+. Compared to other measured fluxes, wastewater discharge is a major contributor of NH_4^+ to the CFRE.

DISCUSSION

Fluxes of nitrogen in the CFRE were determined for individual nitrogen species and for total nitrogen. In order to determine fluxes, the estuary was considered as a box encompassing an area and volume of 1×10^8 m^2 and 9×10^{11} L, respectively (Shank et al., 2004), extending from Navassa at the head of the estuary to M18 at the mouth. Major net inputs and exports have been compiled for each nitrogen species by season (Figure 5).

This model considers five measured nitrogen sources (five for NH_4^+; four for amino acids and TN; and three for NO_3^- and DON,) and two sinks. The sources include

Table 10. Seasonal summary of nitrogen species measured in wastewater treatment delivered to the Cape Fear River Estuary 2002-2003. Average concentrations are µmoles L^{-1} and indicated by an asterisk and fluxes are in mols $season^{-1}$. The Northside Treatment plant is upstream from downtown Wilmington and the Southside Treatment plant is located downstream from Wilmington, near M54. ON = organic nitrogen and TKN = total Kjeldahl nitrogen from unfiltered samples.

Northside							
Season	**Discharge** ($L\ d^{-1}$)	**NH_4^+**		**ON**		**TKN**	
		*µmoles L^{-1}	mols $season^{-1}$	*µmoles L^{-1}	mols $season^{-1}$	*µmoles L^{-1}	mols $season^{-1}$
Spring	2.7×10^7	1500	3.7×10^6	300	7.0×10^5	1800	4.4×10^6
Summer	2.9×10^7	1100	2.9×10^6	300	8.0×10^5	1400	3.7×10^6
Fall	2.5×10^7	1000	2.3×10^6	460	1.1×10^5	1500	3.4×10^6
Winter	2.2×10^7	1300	2.6×10^6	390	8.0×10^5	1700	3.4×10^6
Southside							
Season	**Discharge** ($L\ d^{-1}$)	**NH_4^+**		**ON**		**TKN**	
		*µmoles L^{-1}	mols $season^{-1}$	*µmoles L^{-1}	mols $season^{-1}$	*µmoles L^{-1}	mols $season^{-1}$
Spring	3.4×10^7	1000	3.2×10^6	630	5.2×10^6	1700	5.2×10^6
Summer	3.6×10^7	1200	3.8×10^6	440	5.2×10^6	1600	5.2×10^6
Fall	3.4×10^7	1200	3.6×10^6	230	4.3×10^6	1400	4.3×10^6
Winter	3.1×10^7	1200	3.3×10^6	520	4.7×10^6	1700	4.7×10^6
Combined							
Season	**Discharge** ($L\ d^{-1}$)	**NH_4^+**		**ON**		**TKN**	
		*µmoles L^{-1}	mols $season^{-1}$	*µmoles L^{-1}	mols $season^{-1}$	*µmoles L^{-1}	mols $season^{-1}$
Spring	6.1×10^7	1300	6.9×10^6	400	2.7×10^6	1700	9.6×10^6
Summer	6.5×10^7	1200	6.7×10^6	300	2.2×10^6	1500	8.9×10^6
Fall	5.9×10^7	1100	5.9×10^6	300	1.8×10^6	1500	7.7×10^6
Winter	5.3×10^7	1200	5.9×10^6	500	2.2×10^6	1700	8.1×10^6
Annual	**2.4×10^7**	**1200**	**2.5×10^7**	**400**	8.9×10^6	**1600**	**3.4×10^7**

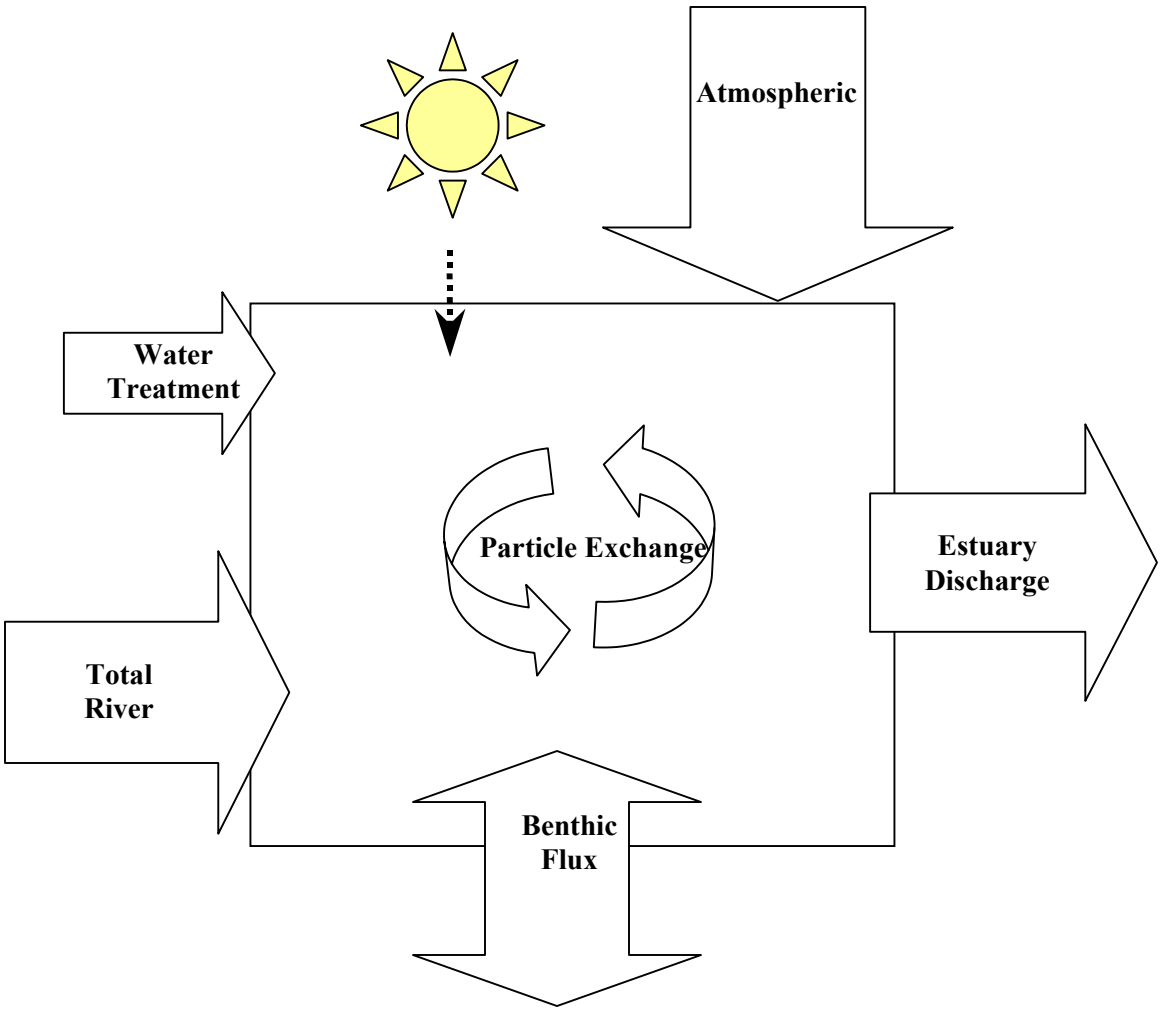


Figure 5. Box model for the Cape Fear River Estuary.

riverine input, wastewater treatment discharge, sediment-water exchange, and atmospheric deposition (only wet precipitation). The two nitrogen sinks measured for this study were sediment uptake (benthic fluxes into the sediment) and estuary discharge to the coastal sea.

Ammonium

Rivers

River fluxes were the largest source of NH_4^+ (7.0×10^6 to 1.4×10^7 mols·season^{-1}) in all seasons except winter (Table 11). The largest flux occurred in spring when rivers contributed >55% to the spring flux of NH_4^+. This was similar to observed inputs into the Scheldt Estuary, Netherlands, where 60% of NH_4^+ came from rivers (Middelburg and Nieuwenhuize, 2001).

Wastewater Treatment

Fluxes of NH_4^+ in wastewater treatment discharge (5.9×10^6 to 6.9×10^6 mols·season^{-1}) did not vary seasonally, although the largest contribution occurred in fall when >40% of the total NH_4^+ input to the CFRE came from wastewater treatment. The lack of seasonality is reasonable because the Northside and Southside facilities control the discharge volume (5.3×10^7 to 6.5×10^7 L·d^{-1}; Table 10) to the estuary. This volume of wastewater discharge is <1% of the river volume into the CFRE so it appears that wastewater would have an insignificant impact on the estuary. However, the distribution of NH_4^+ in the CFRE (Figure 6) indicates there is an NH_4^+ source in the upper estuarine area with relatively conservative mixing usually occurring along the remainder of the salinity gradient. This peak in concentrations may represent wastewater treatment input

Table 11. Ammonium fluxes in moles per season. Negative values indicate a loss from the Cape Fear River Estuary. Percent gain in the table indicates the % of input retained or taken up in the estuary relative to estuarine discharge.

NH_4^+					
Source	**Spring**	**Summer**	**Fall**	**Winter**	**Annual**
River	1.4×10^7	9.4×10^6	7.0×10^6	7.2×10^6	$\mathbf{3.8 \times 10^7}$
Wastewater Treatment	6.9×10^6	6.7×10^6	5.9×10^6	5.9×10^6	$\mathbf{2.5 \times 10^7}$
Benthic Flux	2.1×10^6	2.6×10^6	-1.0×10^6	9.0×10^6	$\mathbf{1.3 \times 10^7}$
Atmospheric	5.1×10^5	3.7×10^5	1.7×10^5	9.1×10^4	$\mathbf{1.1 \times 10^6}$
Particle Exchange	4.3×10^5	2.8×10^5	1.8×10^5	2.8×10^5	$\mathbf{1.2 \times 10^6}$
Total input	2.4×10^7	1.9×10^7	1.4×10^7	2.2×10^7	$\mathbf{7.9 \times 10^7}$
Estuary Discharge	-1.6×10^7	-1.0×10^7	-5.3×10^6	-4.8×10^6	$\mathbf{-3.6 \times 10^7}$
Δ Net Flux	8.0×10^6	9.0×10^6	8.7×10^6	1.7×10^7	$\mathbf{4.3 \times 10^7}$
% gain	33%	47%	62%	78%	**54%**

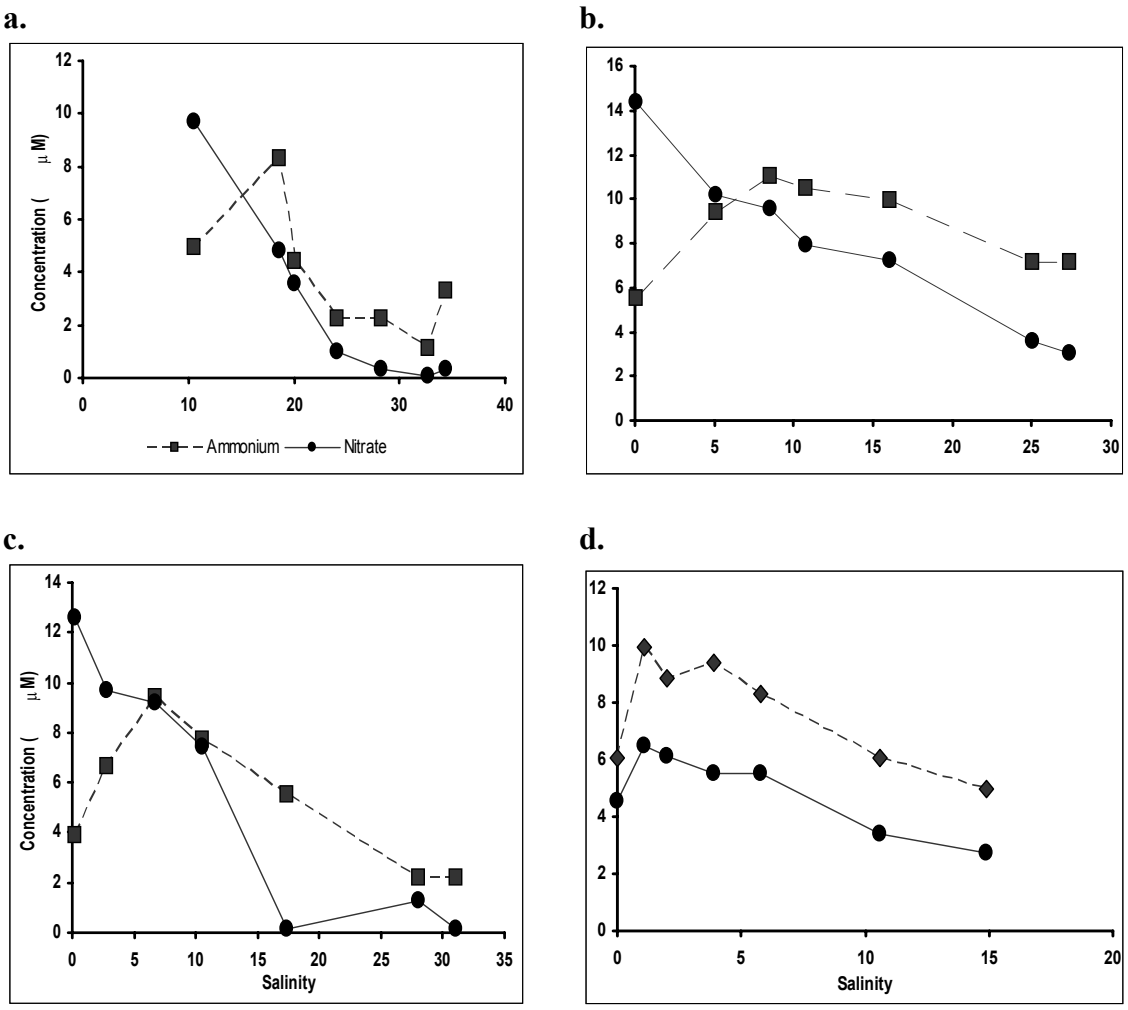


Figure 6. Distributions of NH_4^+ and NO_3^- in the Cape Fear River Estuary over the range of salinities from Navassa to M18. Data from Mallin et al. (1995-2003). **a.)** July 2002 **b.)** November 2002 **c.)** February 2003 **d.)** May 2003

because the percent increase (40 -60%) between concentrations of NH_4^+ first entering the CFRE and the concentration NH_4^+ increases to in the upper estuary is similar to the percent contribution (27-44%) by wastewater treatment discharge toward total NH_4^+ input suggesting that peaks of NH_4^+ in the upper CFRE may result at least partly from wastewater discharge. The input of wastewater NH_4^+ to the CFRE is similar to that observed by Middelburg and Nieuwenhuize (2001), who reported that 40% of NH_4^+ in the Scheldt Estuary, Netherlands was derived from wastewater.

Benthic Fluxes

To determine the effect of benthic fluxes on the entire estuary, fluxes from each site were used in the following calculation:

$$Mols \cdot season^{-1} = flux\ (mols \cdot m^{-2} \cdot d^{-1}) \times CFRE\ area\ (m^2) \times \%\ of\ CFRE \times 90\ (d \cdot season^{-1}),$$

where *flux* = average seasonal fluxes measured from three sites in the estuary and *% of CFRE* = 10% for fluxes at M61, and 90% for the average of fluxes from Sta. 1 and Sta. 2. These area are those estimated to contain sediment types best represented by the measured fluxes at stations in the upper (M61) and lower (Sta. 1 and 2) estuary. In general, benthic fluxes were a net source of NH_4^+ (-1.0 x 10^6 to 9.0 x 10^6 mols·season^{-1}) to the estuary except in fall when there was net uptake by sediments, contributing >10% to the loss of NH_4^+ from the CFRE. The largest benthic fluxes of NH_4^+ occurred in winter, supplying >35% to the total net flux of NH_4^+ to the CFRE in winter. A previous study of benthic fluxes from intertidal sediments near the mouth of the CFRE, (Tzannis, 2000) found that fluxes from sandy sediments with muddy peat were largest in winter (40 μmols·m^2·d^{-1}) and fluxes from muddy sand sediments were largest in spring (85

μmols·m^2·d^{-1}). It was also determined in that study that sediment fluxes may provide 20-40% of the riverine input of NH_4^+.

Many other studies in estuaries and shallow coastal areas report low NH_4^+ fluxes from sediments in winter, with larger releases in spring and summer relative to the rest of the year (Aller and Benninger, 1981; Banta et al., 1995; Boynton and Kemp, 1985; Burdige and Zheng, 1998; Clavero et al., 2000; Cowan and Boynton, 1996; Fisher et al., 1982; Kemp et al., 1990; Laursen and Seitzinger, 2002; Teague et al., 1988; Wilson and Brennan, 2004). Several of these authors suggest a correlation between seasonal variations in NH_4^+ fluxes and sediment temperature variations (Aller and Benninger, 1981; Burdige and Zheng, 1998; Cowan and Boynton, 1996; Fisher et al., 1982; Kemp et al., 1990; Teague et al., 1988; Wilson and Brennan, 2004). In the South River Estuary, North Carolina, benthic fluxes of NH_4^+ were positively correlated with temperature. When temperatures were >15 ºC, NH_4^+ fluxed from sediments (5300 μmols·m^2·d^{-1}), and at temperatures <15 ºC, NH_4^+ fluxes were negligible (0 μmols·m^2·d^{-1}) (Fisher et al., 1982). However, this was not observed in the CFRE. The largest fluxes occurred in winter (610 to 6100 μmols·m^2·d^{-1}) when temperatures were typically low (7.4 to 8.4 ºC) and in summer, when benthic fluxes were smaller (-100 to 3300 μmols·m^2·d^{-1}), temperatures were highest for the year (25.5 to 27.3 ºC) (Table 1).

Temperature was significantly correlated with some but not all nutrient fluxes from silty clay and sand sediments measured by Cowan and Boynton (1996) in Chesapeake Bay. They suggested that NH_4^+ production in sediments may have increased in spring due to increasing organic matter supply and decomposition rates in sediments. Fear et al. (2004) made similar observations in the Neuse River Estuary, attributing NH_4^+

fluxes to the quality and quantity of organic matter in shallow and deep estuarine sediments. Similarly, in the CFRE, the largest fluxes were from the most organic-rich sediments. In Waquoit Bay, Massachusetts, Kirkpatrick et al. (1998) observed larger rates of NH_4^+ and total DIN fluxes from estuarine sediments receiving increased inputs of nitrogen than from control sediments which were from a pristine, low nutrient marsh site, suggesting that nitrogen loading causes increased rates of nitrogen fluxes and faster nitrogen cycling in estuary sediments. This is supported by Boynton et al. (1995) who reported a significant relationship between TN loading and benthic fluxes of NH_4^+ from silty clay and sand Chesapeake Bay sediments.

Lack of sunlight exposure may also play an important role in the magnitude of NH_4^+ fluxes. In the Neuse River Estuary, Rizzo et al. (1992) observed significantly different fluxes from sandy sediments under dark and light treatments. When sediments were kept in the dark, or at very low light levels, there were generally significant NH_4^+ releases (300 to 530 $\mu mols \cdot m^2 \cdot d^{-1}$), whereas uptake (-260 to -72 $\mu mols \cdot m^2 \cdot d^{-1}$) occurred when sediments were exposed to greater levels of light. The CFRE is a light-limited estuary (Mallin et al., 1999a) and bottom sediments are rarely exposed to sunlight, suggesting limited uptake by benthic phytoplankton.

Benthic fluxes of NH_4^+ in the CFRE were smaller (-320 to 6100 $\mu moles\ NH_4^+ \cdot m^{-2} \cdot d^{-1}$) than those reported for Chesapeake Bay (850 to 19700 $\mu moles\ N \cdot m^{-2} \cdot d^{-1}$) (Boynton and Kemp, 1985) and for the Neuse River Estuary (1700 to 11000 $\mu mols\ m^{-2} d^{-1}$) but were similar to fluxes in the South River Estuary, North Carolina (0 to 6400 $\mu mols\ m^{-2} d^{-1}$) (Fisher et al., 1982). The Neuse (55 $m^3 \cdot s^{-1}$) and South River Estuaries (1 $m^3 \cdot s^{-1}$) have

smaller average freshwater inputs than the CFRE (252 $m^3 \cdot s^{-1}$), but similar organic-rich sediments.

Generally, NH_4^+ is the dominant form of DIN fluxing from sediments (Banta et al., 1995; Burdige and Zheng, 1998; Hopkinson et al., 1999; Wilson and Brennan, 2004). In Buzzards Bay Massachusetts, NH_4^+ fluxes (1000 to 10000 $\mu mols \cdot m^2 \cdot d^{-1}$) from silt-clay and muddy sediments were 50% larger than DIN fluxes (500 to 4500 $\mu mols \cdot m^2 \cdot d^{-1}$) (Banta et al., 1995); and in silt-clay organic-rich sediments from Plum Island Sound Estuary, MA, DIN (300 to 65000 $\mu mols \cdot m^2 \cdot d^{-1}$) was >90% NH_4^+ (Hopkinson et al., 1999). In the CFRE, NH_4^+ dominates DIN fluxes (570 to 3100 $\mu mols \cdot m^2 \cdot d^{-1}$) in summer and winter (Table 3) and contributes >80% to the annual DIN flux.

Atmospheric Fluxes

Atmospheric fluxes were calculated using data collected by Long (2003) and the following equation:

$$Mols \cdot season^{-1} = C \times V\ (m \cdot season^{-1}) \times CFRE\ (m^2) \times 1000\ L \cdot m^{-3},$$

where C = the seasonal volume weighted average (VWA) and V = the total volume of wet precipitation collected for the season. There was seasonal variation in the atmospheric flux of NH_4^+ with the smallest flux occurring in winter and the largest in spring (9.1×10^4 to 5.1×10^5 $mols \cdot season^{-1}$). Spring was the only time NH_4^+ was the major component, contributing >55% to TDN. Other studies along the eastern US coast have shown the same pattern of atmospheric fluxes to estuaries (Russell et al., 1998; Whitall et al., 2003). After measuring wet deposition of nitrogen to Chesapeake Bay, Russell et al. (1998) reported the largest NH_4^+ deposition in spring and summer, with the lowest in winter. However, the VWA to Chesapeake Bay was higher (13.6 µM) than in

this study (7 μM) and the average daily flux of NH_4^+ was considerably larger (290 μmols $m^{-2}d^{-1}$) than that observed for the CFRE (29 μmols $m^{-2}d^{-1}$). Russell et al. (1998) suggested the spring/summer maximum of NH_4^+ concentrations may be a result of warmer weather resulting in increasing soil, fertilizer and animal excreta emissions. In the Neuse River estuary, Whitall et al. (2003) also observed highest NH_4^+ concentrations in spring, but reported a generally even distribution between NH_4^+, NO_3^- and DON. Atmospheric deposition may provide significant episodic additions of NH_4^+ to the CFRE but is relatively unimportant on an annual basis.

Exchangeable Ammonium

Ammonium adsorption seems to be most important in the top few centimeters of sediments in dynamic environments such as shallow coastal waters (Van Raaphorst and Malschaert, 1995). Desorption of NH_4^+ following storm and tide-generated resuspension can, in a short amount of time, enrich overlying water (Fanning et al., 1982). Fluxes were calculated to determine the seasonal effect of suspended sediment and particle exchange in the CFRE. However, because exchangeable experiments give the maximum exchange that could occur with greatest salinity, concentrations are larger than would be expected under normal estuarine conditions. For this reason, a mixing experiment was performed using unfiltered freshwater and high salinity (S > 20) water from the estuary mouth. The water was mixed at different proportions to simulate an estuarine salinity gradient. Samples were then filtered and analyzed for NH_4^+ and amino acids as usual. The release that occurred between S= 0 to 5 was used in the following equation (Figure 7):

$$Mols{\cdot}season^{-1} = C\ (mols{\cdot}L^{-1}) \times streamflow\ (m^3{\cdot}sec^{-1}) \times \%\ of\ CFRE \times 1000\ L{\cdot}m^{-3} \times 60\ sec{\cdot}min^{-1} \times 60\ min{\cdot}hr^{-1} \times 90\ (d{\cdot}season^{-1}) \times 24\ h{\cdot}d^{-1},$$

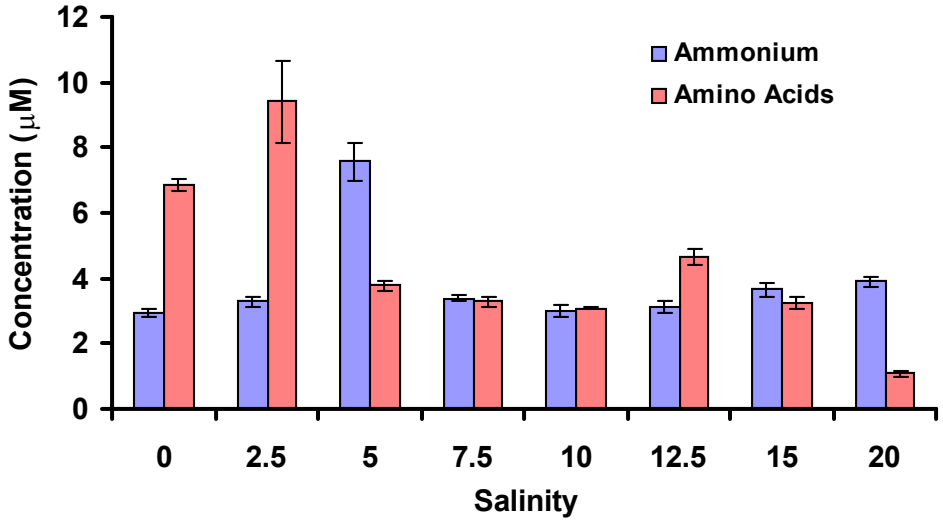


Figure 7. Distribution of NH_4^+ and amino acids for a laboratory-simulated salinity gradient typical to the Cape Fear River Estuary from Navassa to M18.

where C = the average seasonal release of NH_4^+ from suspended particles in low-salinity conditions, s*treamflow* = seasonal average of monthly river flow available from USGS and *% of CFRE* = 0.5% of the estuary volume in which particle exchange is estimated to occur. Particle exchange fluxes (1.8×10^5 to 4.3×10^5 mols·season^{-1}) were a small source of NH_4^+ to the CFRE and were similar in magnitude to atmospheric fluxes, except in winter when particle exchange was a bigger flux.

Estuary Discharge

Export of nitrogen species via estuarine discharge was calculated using the following equation from the United States Geological Survey (USGS):

$$Q_t = Q_1 + Q_2 + Q_3 + Q_4,$$

where Q_t = the total outflow from the estuary, Q_1 = discharge at Lock 1 (Cape Fear River) on the day of Q_t, Q_2 = discharge at Tomahawk (Black River) two days prior to Q_t, Q_3 = discharge at Chinquapin (Northeast Cape Fear River), and Q_4 = runoff estimated using $Q_2 + Q_3$ and figure 2.1 from USGS report (Giese et al., 1979). This information was then used in the following calculation:

$$Mols{\cdot}season^{-1} = Q_t \times C\ (mols{\cdot}L^{-1}) \times 1000\ L{\cdot}m^{-3} \times 60\ sec{\cdot}min^{-1} \times 60\ min{\cdot}hr^{-1} \times 90\ (d{\cdot}season^{-1}) \times 24\ h{\cdot}d^{-1},$$

where C = average seasonal N concentration measured at station M18.

Loss of NH_4^+ from the CFRE through estuarine discharge (5.3×10^6 to 1.6×10^7 mols·season^{-1}) was always smaller than the total NH_4^+ input to the estuary. The percent difference between inputs and exports was determined using the following equation:

$$\%\ difference = [(Flux_{in} - Flux_{out})/Flux_{in}] \times 100$$

where $Flux_{in}$ = the total measured input of N to the CFRE and $Flux_{out}$ = total export of N via estuarine discharge. For NH_4^+, the difference was always positive (33 to 78%, with the largest occurring in winter), indicating the input is retained or taken up in the estuary relative to estuarine discharge. Although biological uptake of nitrogen species may occur in the water column or along the margins and marshes of the estuary, no direct measurements of such uptake were made in this study.

Nitrate

Rivers

Fluxes for NO_3^- were calculated as described previously for NH_4^+ and are summarized in Table 12. As for NH_4^+, river fluxes of NO_3^- were its largest source to the CFRE. There was not a seasonal difference in fluxes, however the largest riverine contribution occurred in winter with >90% of NO_3^- coming from the rivers. River concentrations in this study (1 to 16 μM) were lower than reported for other systems. In Fourleague Bay, Lousiana, riverine concentrations of NO_3^- reached 180 μM with seasonal variations associated with river discharge. The highest river discharge occured in spring (10000 $m^3 \cdot s^{-1}$) and the lowest was normally in fall-winter (2000 $m^3 \cdot s^{-1}$) (Teague et al., 1988). Fourleague Bay is an estuary with high rates of river discharge, whereas river discharge to the CFRE (160 to 370 $m^3 \cdot s^{-1}$, during this study period) is much smaller but closer to that reported for other systems. The Humber Estuary in England has a similar, but more variable freshwater input from the River Ouse (<10 in summer to > 400 $m^3 \cdot s^{-1}$ in winter), and considerably larger NO_3^- concentrations (< 550 μM) (Uncles et al., 1998a). Such large concentrations of NO_3^- may be explained by agricultural runoff

Table 12. Nitrate fluxes in moles per season. Negative values indicate a loss from the Cape Fear River Estuary. Percent gain in the table indicates the % of input retained or taken up in the estuary relative to estuarine discharge.

NO_3^-					
Source	**Spring**	**Summer**	**Fall**	**Winter**	**Annual**
River	2.2×10^7	1.5×10^7	1.7×10^7	2.1×10^7	$\mathbf{7.5 \times 10^7}$
Benthic Flux	3.5×10^6	3.1×10^5	5.8×10^6	8.0×10^5	$\mathbf{1.0 \times 10^7}$
Atmospheric	3.5×10^5	5.7×10^5	2.2×10^5	1.8×10^5	$\mathbf{1.3 \times 10^6}$
Total In	2.6×10^7	1.6×10^7	2.3×10^7	2.2×10^7	$\mathbf{8.7 \times 10^7}$
Estuary Discharge	-1.1×10^7	-4.2×10^6	-2.3×10^6	-2.6×10^6	$\mathbf{-2.0 \times 10^7}$
Δ Net Flux	1.5×10^7	1.2×10^7	2.1×10^7	1.9×10^7	$\mathbf{6.7 \times 10^7}$
% gain	58%	74%	90%	88%	**77%**

(Jarvie et al., 1997). Nitrate was the major component (95%) of riverine DIN to Chesapeake Bay (Boynton et al., 1995), with largest concentrations observed in spring and winter (50 μM). Riverine DIN discharged to Plum Island Sound Estuary, MA was also primarily NO_3^- (90%), (Hopkinson et al., 1999) with concentrations (7 to 30 μM) similar to this study (4 to 22 μM, >50% as NO_3^-).

Wastewater Treatment

Nitrate was not measured in wastewater treatment discharge, however in order to determine if there is any unmeasured NO_3^- in wastewater treatment that may affect the total NO_3^- input to the estuary, a wastewater discharge flux was determined using measured values for NH_4^+ as an upper limit of possible NO_3^- from wastewater. When wastewater treatment fluxes for NO_3^- calculated this way are included, the total input of NO_3^- to the CFRE increased by <10%. The following calculation from was also used to determine if there is any N unaccounted for in the measurements of wastewater discharge by considering the estimated amount of N per person generally found in wastewater treatment:

$$\textit{Mol N in wastewater}\cdot\textit{season}^{-1} = \textit{5 kg N}\cdot\textit{person}^{-1}\cdot\textit{y}^{-1} \times \textit{125,000 persons} \times \textit{1000}$$

$$\textit{g}\cdot\textit{kg}^{-1} \times \textit{1 mol N} \times \textit{14 g} \times \textit{0.25 y}\cdot\textit{season}$$

where $\textit{5 kg N}\cdot\textit{person}^{-1}\cdot\textit{y}^{-1}$ = estimated N content in wastewater treatment based on population Wanielista and Yousef (1993) and *125,000 persons* = estimated population of Wilmington and surrounding area whose sewage is treated by Wilmington facilities. This gives 1.1×10^7 mols $N\cdot season^{-1}$ in wastewater treatment which is within 10 to 30% of the fluxes calculated from measured N in wastewater, suggesting that there is a small amount of unmeasured NO_3^- in wastewater treatment. Further evidence that NO_3^- addition from

wastewater discharge is not important is apparent from mixing diagrams of the CFRE. Nitrate is generally conservatively mixed in the CFRE (Figure 5), except for some occasions of uptake during summer months. This in contrast to NH_4^+ which is not conservatively mixed, but instead exhibits an increase in concentration in the upper estuary indicative of inputs by wastewater treatment and benthic fluxes from organic-rich sediments.

Benthic Fluxes

Benthic fluxes were a net source of NO_3^- to the CFRE with the largest flux occurring in fall (5.8×10^6 mols ·season^{-1}) when benthic fluxes contributed >20% to the fall influx of NO_3^-. Many authors report small benthic fluxes of NO_3^- (Burdige and Zheng, 1998; Cowan and Boynton, 1996; Kemp et al., 1990; Rizzo et al., 1992), or net uptake of NO_3^- by sediments (Burdige and Zheng, 1998; Caffrey et al., 2002; Cook et al., 2004; Kemp et al., 1990; Kirkpatrick et al., 1998). However, Teague et al. (1988) reported similar NO_3^- fluxes (-2688 to 1896 μmols·m^{-2}·d^{-1}) from shallow, fine-grained sediments in Fourleague Bay to those in this study (-600 to 1500 μmols·m^{-2}·d^{-1}).

Atmospheric Fluxes

Atmospheric fluxes of NO_3^- to the CFRE varied seasonally with the smallest flux occurring in winter and the largest in summer (1.8×10^5 to 5.8×10^5 mols NO_3^-·season^{-1}). In the Neuse River Estuary, NO_3^- made up 32% of the annual N deposition to the estuary, with highest concentrations in spring (Whitall et al. 2003). In Chesapeake Bay, Russell et al. (1998) reported the largest NO_3^- concentrations (21.5 μM) in spring and early summer; these were close to the VWA of NO_3^- in this study (15 μM). They suggested the spring/summer peak was due to increased soil emissions of NO_x. Average daily fluxes of

NO_3^- (34 μmols $m^{-2}d^{-1}$) to the CFRE were considerably smaller than fluxes to Chesapeake Bay (460 μmols $m^{-2}d^{-1}$) (Russell et al., 1998).

Estuarine Discharge

Estuarine discharge of NO_3^- was calculated as previously described for NH_4^+. Similarly, loss of NO_3^- from the CFRE through estuarine discharge (1.1 x 10^7 to 2.1 x 10^7 mols·$season^{-1}$) was always smaller than the total NO_3^- input to the estuary. The % difference was also calculated as described for NH_4^+. Likewise, the difference was always positive (58 to 90%, with the largest occurring in fall), again, indicating the input is retained or taken up in the estuary relative to estuarine discharge. Such uptake may be due to biological utilization in the water column or marsh margins in the CFRE, however no such measurements of these processes were made in this study.

Organic Nitrogen

Rivers

Fluxes for ON were calculated as described previously for NH_4^+ and are summarized in Table 13. River fluxes were the largest source of ON to the CFRE in all seasons (1.2 x 10^8 to 1.9 x 10^8 mols·$season^{-1}$), particularly in spring when streamflow was greatest. Organic nitrogen was overwhelmingly the dominant N species in rivers with the largest ON concentration occurring in fall (79 μM). Likewise, DON concentrations in rivers to Chesapeake Bay were largest in summer and fall, and of similar magnitude (60-75 μM) (Magnien et al., 1992). Dissolved ON concentrations in Fourleague Bay, LA were larger and displayed strong seasonality related to river discharge, with largest concentrations in winter (113.1 μM) (Teague et al., 1988).

Table 13. Organic nitrogen (ON) fluxes in moles per season in the Cape Fear River Estuary. Negative values indicate a loss from the system. Percent gain in the table indicates the % of input retained or taken up in the estuary relative to estuarine discharge. An asterisk indicates fluxes of dissolved ON.

ON					
Source	**Spring**	**Summer**	**Fall**	**Winter**	**Annual**
River	1.9×10^8	1.4×10^8	1.2×10^8	1.4×10^8	$\mathbf{5.9 \times 10^8}$
Wastewater	2.7×10^6	2.2×10^6	1.8×10^6	2.2×10^6	$\mathbf{1.1 \times 10^7}$
Benthic Flux*	1.1×10^6	2.2×10^5	-3.6×10^5	-2.9×10^6	$\mathbf{-2.0 \times 10^6}$
Atmospheric*	0.0	1.1×10^5	1.7×10^5	2.1×10^4	$\mathbf{3.0 \times 10^5}$
Total In	1.9×10^8	1.4×10^8	1.2×10^8	1.4×10^8	$\mathbf{6.0 \times 10^8}$
Estuary Discharge	-1.2×10^8	-1.2×10^8	-6.1×10^7	-4.8×10^7	$\mathbf{-3.5 \times 10^8}$
Δ Net Flux	7.0×10^7	2.0×10^7	5.9×10^7	9.2×10^7	$\mathbf{2.5 \times 10^8}$
% gain	37%	14%	49%	66%	**42%**

Benthic Fluxes

Benthic fluxes were a net sink of DON in the CFRE, with small releases occurring in spring and summer and larger uptake occurring in fall and winter (-3.6 x 10^5 to 1.1 x 10^6 mols·season^{-1}). Similarly, in Plum Island Sound Estuary, MA, Hopkinson et al. (1999) observed a net sink of DON to sediments for most of the year except for few outward fluxes occurring in summer (-9500 to <3000 μmoles·m^{-2}·d^{-1}) and Teague et al., (1988) observed much larger and variable fluxes of DON (-17040 to 7320 μmoles·m^{-2}·d^{-1}) in Fourleague Bay. Fourleague Bay is a shallow estuary (1.5 m) compared to the CFRE (10-15 m) but the sediment types are similar; both estuaries have fine-grained sediments and similar % organic matter in sediments (Fourleague: 3.8%, CFRE: 4.8%). Daily fluxes in this study were small (-1500 to 200 μmoles·m^{-2}·d^{-1}) and similar to reported fluxes from other systems. Fluxes from Chesapeake Bay sediments were a small source of DON (-250 to 550 μmoles·m^{-2}·d^{-1}) (Cowan and Boynton, 1996) and (-10 to 420 μmoles·m^{-2}·d^{-1}) (Burdige and Zheng, 1998) but DON was considered to be an important component of TN fluxes in spring and early summer (Cowan and Boynton, 1996). Fluxes of DON in tidally-influenced sediments from Bremer River, Australia were insignificant in winter (when water column N concentrations were highest), but considerable in both directions during summer (-2520 to 936 μmoles·m^{-2}·d^{-1}), with DON releases associated with NO_3^- and NH_4^+ consumption (Cook et al., 2004). DON fluxes in Bremer River were at least twice as large as DON fluxes in this study, and where DON release was associated with DIN consumption, the opposite appears to be true in the CFRE: DIN releases are associated with DON consumption (Table 3).

Atmospheric Fluxes

Atmospheric fluxes of DON were the smallest of measured DON fluxes and there was no apparent seasonality. No flux was observed in spring and the largest flux occurred in fall (0 to 2.1 x 10^4 mols·season^{-1}). Atmospheric VWA and daily fluxes of DON at Chesapeake Bay were larger (6.2 µM, 170 µmoles·m^{-2}·d^{-1}) than in this study (1.6 µM, 7.7 µmoles·m^{-2}·d^{-1}) and were largest in spring (Russell et al., 1998). Fluxes from both systems are comparatively low but similar in contribution to N in atmospheric deposition, making up 13% of TN in Chesapeake Bay and 12% of TDN in the CFRE.

Estuarine Discharge

Estuarine discharge of ON was calculated as previously described for NH_4^+ and NO_3^-. Similarly, loss of ON from the CFRE through estuarine discharge (4.8 x 10^7 to 1.2 x 10^8 mols·season^{-1}) was always smaller than the total ON input to the estuary. The % difference was also calculated as described for the inorganic N (IN). Likewise, the difference was always positive (14 to 66%, with the largest occurring in winter), again, indicating the input is retained or taken up in the estuary relative to estuarine discharge, however the retention of ON is much smaller than for the IN species.

Amino Acids

Rivers

Fluxes of amino acids were calculated as described previously for NH_4^+ and are summarized in Table 14. Riverine fluxes were the largest amino acid source to the CFRE, although of smaller magnitude than for other N species. There was seasonal variability in fluxes (2.2 x 10^6 to 4.3 x 10^6 mols·season^{-1}), however the largest river contribution

Table 14. Amino acid fluxes in moles per season. Negative values indicate a loss from the Cape Fear River Estuary. Percent gain in the table indicates the % of input retained or taken up in the estuary relative to estuarine discharge and a 'loss' indicates the % of discharge that is greater than input, indicating there is a net source in the estuary.

Amino acids					
Source	**Spring**	**Summer**	**Fall**	**Winter**	**Annual**
River	4.3×10^6	3.4×10^6	2.2×10^6	2.2×10^6	$\mathbf{1.2 \times 10^7}$
Benthic Flux	4.1×10^3	2.3×10^5	4.6×10^4	6.1×10^5	$\mathbf{8.9 \times 10^5}$
Atmospheric	2.8×10^4	4.1×10^4	2.0×10^4	1.2×10^4	$\mathbf{1.0 \times 10^5}$
Particle Exchange	2.3×10^5	9.2×10^4	6.1×10^4	9.6×10^4	$\mathbf{4.8 \times 10^5}$
Total input	4.6×10^6	3.8×10^6	2.3×10^6	2.9×10^6	$\mathbf{1.4 \times 10^7}$
Estuary Discharge	-5.6×10^6	-5.1×10^6	-9.6×10^5	-2.3×10^6	$\mathbf{-1.4 \times 10^7}$
Δ Net Flux	-1.0×10^6	-1.3×10^6	1.3×10^6	6.0×10^5	**0.0**
% loss/gain	-22%	-34%	57%	21%	**0%**

occurred in spring with >90% of amino acids to the CFRE coming from rivers. Fluxes of amino acids made up of 2-5% of ON and 1-2% of TN fluxes in rivers. Concentrations of free amino acids in rivers to the CFRE were also small (1.2 to 2.1 μM), but larger than dissolved free amino acids (DFAA) in rivers to Pearl River Estuary, China (0.15 to 1.1 μM) which made up 7 to 8 % of TN (Chen et al., 2004).

Benthic Fluxes

Benthic fluxes were a small but net source of amino acids (4.1×10^3 to 6.1×10^5 mols·season^{-1}) to the CFRE. There was some seasonality and like NH_4^+, the largest fluxes occurred in winter. Amino acids were <15% of DON in benthic fluxes; similarly, small benthic fluxes of amino acids in Chesapeake Bay made up <5% of the benthic flux of DON (Cowan and Boynton, 1996).

Atmospheric Fluxes

Atmospheric fluxes (1.2×10^4 to 4.1×10^4 mols·season^{-1}) were the smallest source of amino acids to the CFRE. The largest flux occurred in summer when amino acids contributed >35% to the atmospheric deposition of DON. Free amino acid concentrations were also small ranging from 0.03 to 4.6 μM. These are similar to the range reported by Cornell (2003) (0.002 to 6.4 μM) and to the concentration of amino acids in coastal rain (0.61 μM) reported by Mopper and Zika (1987).

Exchangeable Amino Acids

Adsorption to particle surfaces in estuarine systems can be important to the distribution and bioavailability of organic compounds (Gordon and Millero, 1985; Gu et al., 1995; Sugai and Henrichs, 1992; Thimsen and Keil, 1998). Particle exchange fluxes of amino acids were calculated from measurements of amino acid desorption from

particles as described previously for NH_4^+ (Figure 7). Similarly, particle exchange of amino acids (6.1 x 10^4 to 2.3 x 10^5 mols·season^{-1}) was a small source to the CFRE. However, they were larger than atmospheric fluxes in all seasons and similar in magnitude to benthic fluxes in general, with larger fluxes in spring and fall.

Estuarine Discharge

Estuarine discharge of amino acids was calculated as previously described. Loss of amino acids from the CFRE through estuarine discharge (1 x 10^6 to 6 x 10^6 mols·season^{-1}) was smaller than the total amino acid input to the estuary in fall and winter, but larger than the input in spring and summer. The % difference was calculated as described for IN and ON, however the difference was not always positive for amino acids (-34 to 57%, the largest occurred in fall). This indicates that sometimes the input of amino acids were retained or taken up in the estuary relative to estuarine discharge, and sometimes discharge was greater than the input, so there must be a net source of amino acids in the estuary. This may be from biological interconversions of NH_4^+ and NO_3^- to amino acids, especially in spring and summer months in the mouth of the estuary.

Total Nitrogen

Rivers

Fluxes of TN were calculated as described previously for NH_4^+ and are presented in Table 15. Mean seasonal inputs of TN to the CFRE did not vary seasonally. River fluxes were the largest source of TN with the largest flux occurring in spring (2.2 x 10^8 mols N·season^{-1}) when >90% of the spring TN input was from rivers. River fluxes to Chesapeake Bay were also largest in spring (275 mg N·m^{-2}·d^{-1}) although there was

Table 15. Total nitrogen (TN) fluxes in moles per season in the Cape Fear River Estuary. Negative values indicate a loss from the system. Total N was not measured for particle exchange, the value represented is the sum of NH_4^+ and amino acids, also, an asterisk indicates fluxes are total Kejldahl nitrogen and a double asterisk indicates fluxes are total dissolved nitrogen. Percent gain in the table indicates the % of input retained or taken up in the estuary relative to estuarine discharge.

TN					
Source	**Spring**	**Summer**	**Fall**	**Winter**	**Annual**
River	2.2×10^8	1.6×10^8	1.5×10^8	1.7×10^8	$\mathbf{7.0 \times 10^8}$
Wastewater Treatment*	9.6×10^6	8.9×10^6	7.7×10^6	8.1×10^6	$\mathbf{3.4 \times 10^7}$
Benthic Flux**	5.1×10^6	2.9×10^6	3.0×10^6	9.4×10^6	$\mathbf{2.0 \times 10^7}$
Atmospheric**	7.3×10^5	9.9×10^5	5.6×10^5	2.9×10^5	$\mathbf{2.6 \times 10^6}$
Particle Exchange**	6.6×10^5	3.7×10^5	2.4×10^5	3.8×10^5	$\mathbf{1.7 \times 10^6}$
Total input	2.4×10^8	1.7×10^8	1.6×10^8	1.9×10^8	$\mathbf{7.6 \times 10^8}$
Estuary Discharge	-1.5×10^8	-1.3×10^8	-6.9×10^7	-1.1×10^8	$\mathbf{-4.6 \times 10^8}$
Δ Net Flux	9.0×10^7	4.0×10^7	9.1×10^7	8.0×10^7	3.0×10^8
% gain	38%	24%	57%	42%	**39%**

more seasonal variation (84 to 274.8 mg $N \cdot m^{-2} \cdot d^{-1}$) (Boynton et al., 1991, 1992; Baird et al., 1995) than measured in this study of the CFRE (205.1 to 267.4 mg $N \cdot m^{-2} \cdot d^{-1}$). Organic N was always the major component in rivers to the CFRE, contributing >80% to TN in river fluxes. Likewise, in summer and fall, river input of TN to Chesapeake Bay was also >80% DON and particulate nitrogen; but at other times, river concentrations were dominated by DIN (75%, mostly as NO_3^-) (Boynton et al., 1995).

Riverine inputs of N tend to be more important for estuaries that are rapidly flushed (Balls, 1994), such as the CFRE. Ensign et al. (2004) used digital bathymetric data to calculate the flushing time of the CFRE. They calculated short residence times for the CFRE resulting from the large freshwater inflow to the estuary. The residence times vary with season (1-22 days) with the shortest time in winter and longest in summer. Consequently, although the CFRE is continually resupplied with river-borne nutrients, only 9% of riverine nitrogen is used in primary production and recycled in the estuary (Ensign et al., 2004). Therefore, even though river fluxes are the largest source of TN, as a result of the short residence time, riverine nitrogen may have little impact on the estuary, and other estuarine sources may be at least or more qualitatively important to nitrogen cycling in the CFRE as river fluxes.

Wastewater Treatment

Wastewater discharge fluxes of TKN were important to the contribution of TN to the CFRE with the largest fluxes occurring in spring and summer (8.1×10^6 to 9.6×10^6 $mols \cdot season^{-1}$). However, the discharge flux of TKN was <8% of TN input to the CFRE. This is smaller than the range of point source contributions of TN to different systems in Chesapeake Bay which ranged from 9.7% to 48% and were directly proportional to

population densities (Boynton et al., 1995). In this study of the CFRE, NH_4^+ was the major component of wastewater, contributing >65% to the wastewater discharge flux of TKN.

Benthic Fluxes

Benthic fluxes were a net source of TDN to the CFRE in all seasons (2.9 x 10^6 to 9.4 x 10^6 mols·season^{-1}) and the largest flux from sediments occurred in winter when NH_4^+ made up >90% of TDN. Similarly, benthic fluxes of TDN in Fourleague Bay, LA were largest in winter (-13272 to 13032 μmoles·m^{-2}·d^{-1}) and were overall dominated by DON and NO_3^- (Teague et al., 1988). These fluxes were much larger than benthic fluxes in this study of the CFRE (0 to 2700 μmoles·m^{-2}·d^{-1}) which were alternately dominated by $NH_4^+$and NO_3^-. Nitrate was also the dominant form of dissolved N in benthic fluxes from the Bremer River, Australia (Cook et al., 2004).

Atmospheric Fluxes

Atmospheric fluxes were not a major source of N to the CFRE. Whitall et al. (2004) reported that atmospheric N was not a dominant source to four eastern U.S. coast estuaries (Long Island Sound, Chesapeake Bay, Delaware Bay, Pamlico Sound) but did contribute 8 to 22% of the total N load to each estuary. Also, Boynton et al (1995) estimated a similar range of atmospheric inputs (5 to 13%) to Chesapeake Bay, and Whitall et al. (2003) reported that N in wet deposition to the Neuse River Estuary is an important component of the N flux to the watershed, accounting for 15 to 55% of the total 'new' N flux to the estuary.

In this study of the CFRE, seasonal (2.9 x 10^5 to 9.9 x 10^5 mols·season^{-1}) and daily atmospheric fluxes (30 to 100 μmoles·m^{-2}·d^{-1}) were largest in summer and smallest

in winter and were overwhelmingly dominated by DIN (>90% of TDN). Daily fluxes to the Neuse River Estuary (153 to 265 μmoles·m^{-2}·d^{-1}) were also largest in summer and smallest in winter, but were considerably larger than in this study, and were evenly distributed between DIN and DON species.

Estuarine Discharge

Estuarine discharge of TN was calculated as previously described for NH_4^+. Similarly, loss of TN from the CFRE through estuarine discharge (6.9 x 10^7 to 1.5 x 10^8 mols·$season^{-1}$) was always smaller than the TN input to the estuary. The % difference was also calculated as described for NH_4^+. Likewise, the difference was always positive (24 to 57%, with the largest occurring in fall), again, indicating the input is retained or taken up in the estuary relative to estuarine discharge. However, we made no measurements of biological uptake in the water column or marsh margins in the CFRE.

SUMMARY AND CONCLUSIONS

Annual loading of total dissolved N into the CFRE is summarized in Figure 5. Rivers were the dominant source to the estuary (6.9 x 10^8 mols·yr^{-1}) where they contributed >90% to TN loading. The main component was ON making up >80% of TN. The remaining N in rivers was inorganic, comprised primarily by NO_3^- (>10%) with lesser amounts of NH_4^+ (>5%). Wastewater treatment facilities were a secondary source (3.7 x 10^7 mols·yr^{-1}) making up <5% of the annual N input, and were primarily made up of NH_4^+ (>70%). The wastewater treatment and river numbers should be viewed as a maximum input of TDN because it comprises both particulate and dissolved N species.

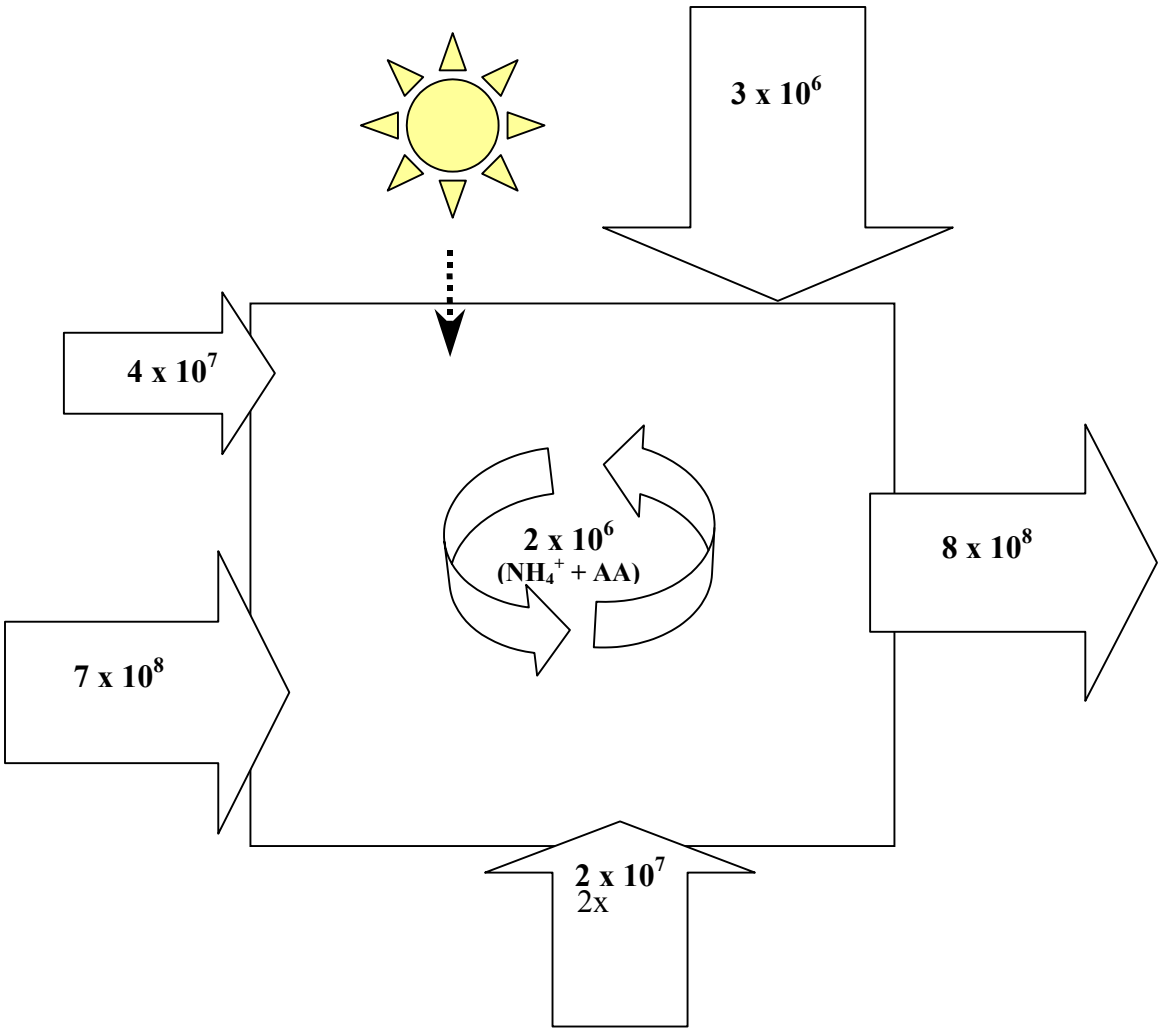


Figure 8. Annual loading of total nitrogen into the Cape Fear River Estuary (moles per year).

Benthic fluxes were a net source (2.3 x 10^7 mols·yr^{-1}) of N to the CFRE, but were relatively small (<3%) compared to rivers. They were primarily made up of inorganic N (>90%), mainly NO_3^- (>35%) and NH_4^+ (>60%). Atmospheric fluxes were in general a minor source (2.6 x 10^6 mols·yr^{-1}) to the CFRE, making up <1% of the annual input of N. Atmospheric deposition is dominated by IN with > 45% present as NO_3^- and >40% as NH_4^+. Organic N makes up the remaining N and is comprised of amino acids which make up >40% of the ON. A minor source of N to the CFRE is particle exchange. Ammonium and amino acids were the only N species measured for particle exchange, so the sum of these species is used to consider the relative importance of each to N fluxes from this source. Particle exchange fluxes (1.9 x 10^6 mols·yr^{-1}) were the smallest of all fluxes, contributing <0.5% to total N loading.

Discharge of estuarine waters to the coastal sea was the primary loss of N from the estuary. The quantity of N leaving the CFRE (4.5 x 10^8 mols·yr^{-1}) via this output was >60% of the annual input of N, and was primarily made up of ON (>75%). This observed imbalance of TN input and exports may have two possible explanations: there might be biological or chemical loss in the system, although no data demonstrates this; or that the input and export are balanced, but there are uncertainties in these measurements (i.e. N flux calculations are a combination of dissolved N measurements and dissolved plus particulate measurements).

In conclusion, river fluxes were overwhelmingly the largest source of each N species to the CFRE. Increasing NH_4^+ concentrations in the CFRE in these river sources is the most likely explanation for the observed increasing NH_4^+ concentrations in the estuary; primarily because rivers were overwhelmingly the dominant source of NH_4^+ for

the year. Also, data from Mallin et al. (1996-2003) shows that while NH_4^+ concentrations are increasing in the CFRE (Figure 1), they are increasing at the same rate in the tributary rivers (Figure 9) and the other N species are decreasing or changing very little (Figure 10). Such an increase in rivers may be due to increasing municipal, industrial, and agricultural inputs within the watershed.

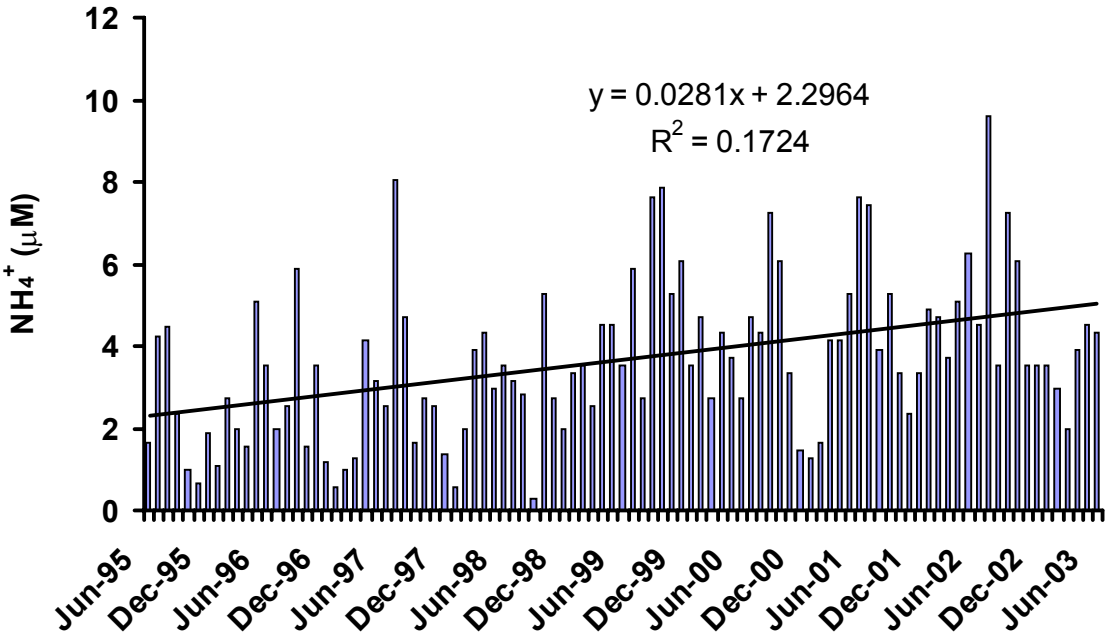
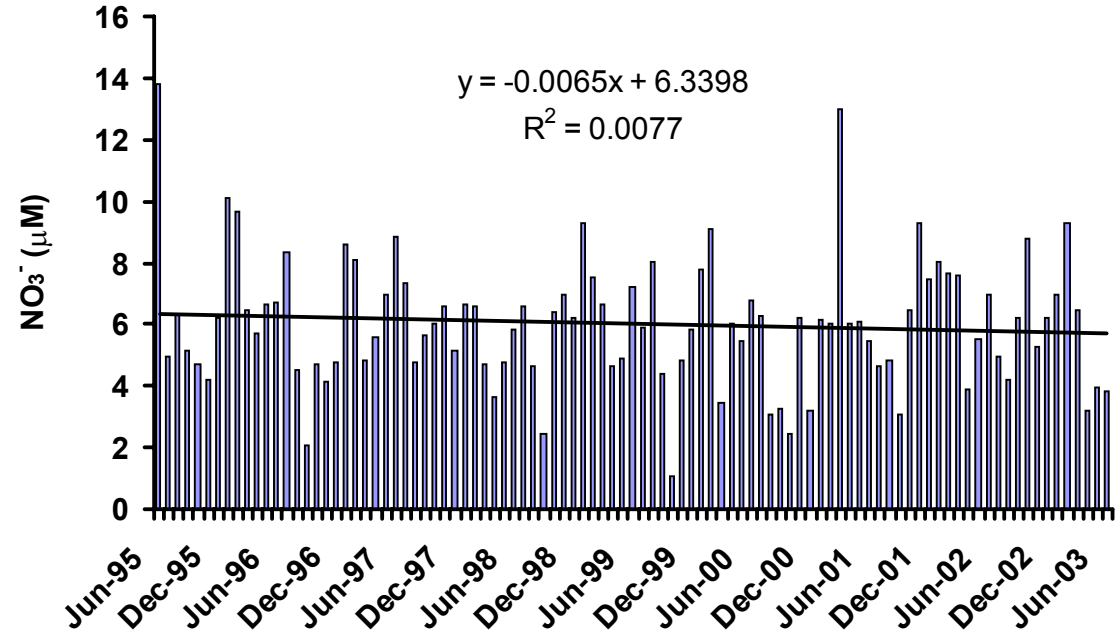


Figure 9. Concentrations of NH_4^+ from monthly data in tributary rivers to the Cape Fear River Estuary from 1995 to 2003 showing a statistically significant increase (t-test, $p \leq 0.05$). Data from Mallin et al. (1996-2003).

a.



b.

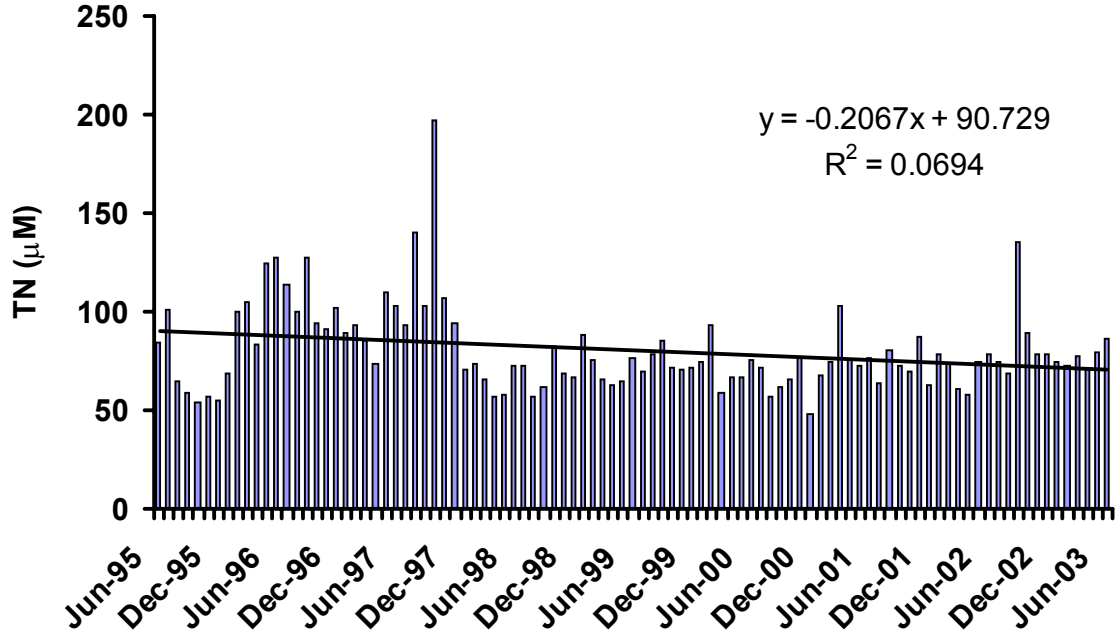


Figure 10. Monthly data in tributary rivers to the Cape Fear River Estuary from 1995 to 2003 showing there is no statistically significant change (t-test, $p \leq 0.05$) in NO_3^- concentrations and there is a statistically significant decrease in total nitrogen (TN) concentrations. Data from Mallin et al. (1996-2003). **a.)** NO_3^- **b.)** TN